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Effects of Diabetes on the Dietary Lipid Alteration of R3230AC Mammary Carcinoma Growth in Rats[1] (42071)

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Abstract. To examine the effects of diabetes on the alteration of R3230AC mammary tumor growth by dietary lipids, streptozotocin-induced diabetic rats were fed diets containing either 20% corn oil (HF), 20% hydrogenated cottonseed oil (HCTO), or 0% fat (FF). Diabetes resulted in lower tumor weights and body weights compared to those of intact animals. Unlike intact animals, relative tumor weight (g tumor/100 g body wt) of diabetic animals fed HF diets were not greater than those from animals fed FF diets. However, in these diabetic animals, growth of tumors in HF-fed rats was faster than in HCTO-fed rats, a relationship similar to that seen in intact rats. A surprising result was the almost twofold greater tumor weight/100 g body wt observed in diabetic FF-fed rats compared to those fed HCTO diets. Insulin binding to tumor plasma membranes from diabetic animals was higher in rats fed HF diets than in rats fed FF or HCTO diets. The tumor plasma membrane fatty acid composition of diabetic rats fed FF and HCTO diets displayed higher proportions of the monounsaturates (C18:1 and C21:1) and decreased amounts of the polyunsaturates (C18:2 and C20:4) compared to the levels observed in membranes from HF-fed rats. These results, as well as the insulin binding data, were similar to those obtained using intact animals. The data presented here indicate that the more rapid growth of the R3230AC mammary tumor seen in intact animals fed high polyunsaturated fat vs fat-free diets did not occur in diabetic animals.

Although it has been demonstrated that dietary lipids can influence the growth and development of mammary tumors, the mechanism by which they act remains largely unknown. Mechanisms involving hormonal milieu (1), hormone binding (2), prostaglandin levels (3–5), and cell-mediated immunity (6), as well as alterations in membrane transport (7–9) and intercellular communication (10), have been examined. We have previously (11) suggested a role for estrogens and insulin in the dietary lipid alteration of mammary tumor growth. This conclusion was based on our observations of a relationship between tumor growth and insulin binding capacity of the tumor plasma membranes. In order to further evaluate the role of insulin and insulin binding in this phenomenon, we have examined the effects of diabetes on the ability of dietary lipids to alter tumor growth.

No previous work, to our knowledge, has addressed the possible relationship between host insulin status and the response of mammary tumors to dietary lipid intake. The tumor model that we selected, the R3230AC transplantable mammary carcinoma, is a hormone-responsive tumor, which has been shown to grow faster in diabetic than in intact rats (12, 13). This tumor has also demonstrated more rapid growth in rats fed high polyunsaturated fat diets compared to those fed fat-free diets (3), and was therefore considered well suited for this study.

The results presented here provide further support for a suggested role of insulin in the dietary lipid alteration of tumor growth.

Materials and Methods. *Animals, tumors, and diets.* Female Fischer rats (75–90 g) were obtained from Charles River Breeding Laboratory (Wilmington, Mass.). The R3230AC tumor was implanted sc in the axillary region on both sides by a sterile trochar technique as described by Hilf *et al.* (14). When the effects of diabetes were studied, diabetes was

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induced 3 days prior to tumor implantation by ip injection of streptozotocin (60 mg/kg), which had been freshly dissolved in 0.9% NaCl solution (30 mg/ml) and was rapidly adjusted to pH 4.5 with 0.25 *M* citric acid. Two days later, urinary glucose was estimated by Clinistix (Ames Co., Elkhart, Ind.). Animals with urinary glucose levels of less than 0.5% (approximately 30% of them) were reinjected with a second dose of streptozotocin. These animals were then equally distributed among the previously injected animals, which had been divided into three equal groups. Animals were then placed on the various diets (Table I); either a FF diet containing 0% fat, a HF diet containing 20% corn oil, or a HCTO diet containing 20% hydrogenated cottonseed oil (final concentration less than 0.1% linoleic acid, 13% stearic acid, and 5.7% palmitic acid). All diets, which were offered *ad libitum*, had an equivalent ratio of protein calories to total calories.

Animals were sacrificed by decapitation at 19 to 20 days after tumor transplantation. Animals from the different groups within each experiment were pair-sacrificed, i.e., approximately three diabetic animals per week per diet group and four to five intact animals per week per diet group were sacrificed at the same time. This design equalized variations due to transplantation of tumors from different weekly donors and offered more representative results. Sacrifice of this number of animals at each time allowed us to prepare membranes from fresh, rather than frozen, tumors. At the time of sacrifice, urinary glucose and blood glucose were estimated by Clinistix (Ames Co.) and by Chemstrip bG (Bio-Dynamics Co., Indianapolis, Ind.). Animals with urinary glucose levels above 0.5% and blood glucose levels above 400 mg/dl were classified as diabetic and their tumors were processed for further study. Blood was collected in 15 × 100-mm plastic tubes, centrifuged for 10 min at 1000*g*, and the serum was removed and stored at −76°C until insulin and glucose levels were measured.

Tumor membrane preparation and analysis. Plasma membranes were prepared essentially by the method of Shin *et al.* (15). Freshly excised tumors were homogenized in 4 vol of ice-cold 0.9% NaCl, an equal volume of ice-cold 0.9% NaCl was added, and the homogenate was centrifuged at 4°C at 1000*g* for 10 min. The layer of fat at the top was removed and the supernatant was collected. The pellet was resuspended in an equal volume of 0.9% NaCl, rehomogenized with 15 strokes in a loose-fitting Dounce homogenizer, and centrifuged; the resulting supernatant was combined with the previously collected supernatant. The pooled supernatants were centrifuged at 4°C at 33,000*g* for 7.5 min and the resulting supernatant collected and centrifuged at 4°C at 96,000*g* for 75 min to yield a microsomal pellet. This pellet was resuspended in Hepes buffer, pH 7.4, containing 25 m*M* Hepes, 10 m*M* $NaHCO_3$, 125 m*M* NaCl, 3 m*M* K_2HPO_4, 1 m*M* $MgSO_4$, 1 m*M* $CaCl_2$, and 11 m*M* glucose by passage through a 26-gauge needle attached to a syringe; the resuspended ma-

TABLE I. DIET COMPOSITION (g/kg)

Component	Fat free diet (FF)	(20%) High fat diet (HF)	(20%) Hydrogenated cottonseed oil diet (HCTO)
Corn oil	—	200	—
Hydrogenated cottonseed oil	—	—	200
Casein (vit. free)	179	230	230
Sucrose	220	145	145
Dextrose	263	175	175
Dextrin	263	175	175
Rogers harper vitamin mix	5	5	5
Rogers harper salt mix	50	50	50
Vit. B_{12} (10 μg/ml)	5 ml/kg	5 ml/kg	5 ml/kg
Choline chloride (0.20 g/ml)	15 ml/kg	15 ml/kg	15 ml/kg

terial was then mixed with an equal volume of 66% (w/w) sucrose. The final sucrose concentration was adjusted to 40% (w/w) by appropriate addition of either 66% sucrose or Hepes buffer as determined by a refractometer. The sample was fractionated by flotation through a step gradient containing 25, 33, and 37% sucrose (w/w) at 96,000*g* at 4°C for 7 hr using an SW27 rotor. The material at the 25–33% sucrose interface was collected, diluted with 3 vol of 10 m*M* Tris–HCl (pH 7.4), and centrifuged for 1 hr at 4°C at 96,000*g*. Membranes were then suspended in a small volume (approximately 1 ml) of Hepes buffer by mixing in a syringe to which a 26-gauge needle was attached. The membrane suspensions were kept at 0°C until used.

Protein was determined by the method of Lowry *et al.* (16). 5′-Nucleotidase activity was assayed as described by Morre (17) and was done in order to assess the similarity of the membrane preparations. The 5′-nucleotidase specific activity per milligram protein was the same for plasma membranes prepared from tumors of diabetic animals fed the FF, HCTO, and HF diets (23.4 ± 1.6, 20.8 ± 1.5, and 21.4 ± 1.9 μmole Pi/mg protein/hr, respectively). Plasma membranes were stored at −76°C until analyzed for fatty acid composition. Lipids were extracted by the method of Folch *et al.* (18). Extracted lipids were methylated and analyzed by gas–liquid chromatography according to the method detailed by Cave and Erickson-Lucas (2). Samples were also submitted to Dr. Charles Sweeley, Michigan State University, for confirmation by GLC/mass spectrometry analysis.

Insulin binding to plasma membranes. Crystalline porcine insulin was labeled stoichiometrically with Na ^{125}I, using chloramine-T according to the method of Freychet *et al.* (19). The labeled insulin had a specific activity of 60–260 μCi/μg and was greater than 98% trichloroacetic acid (TCA) precipitable.

The specific binding of ^{125}I-labeled insulin was measured as follows: 90–150 μg membrane protein were incubated with ^{125}I-labeled insulin in 250 μl Hepes buffer containing 5 mg/ml bovine serum albumin and 1 mg/ml bacitracin to minimize insulin degradation. Binding of ^{125}I-labeled insulin was performed in duplicate in 1.5-ml conical microfuge tubes at final insulin concentrations of 10^{-8}, 4×10^{-9}, 8×10^{-10}, and 2×10^{-10} *M*. For each assay, duplicate samples were also incubated in the presence of 10^{-6} *M* unlabeled insulin to obtain and then correct for nonspecific binding. The contents of the tubes were mixed, and after a 1-hr incubation at room temperature (21°C), 1 ml ice-cold 0.9% NaCl was added and the tubes were centrifuged for 4 min in a Beckman 3200 Microfuge. Membranes were washed once again with 1 ml ice-cold 0.9% NaCl, the tubes were drained and blotted, and placed in a Beckman 8000 gamma counter (Beckman Instruments, Inc., Fullerton, Calif.) for measurement of radioactivity.

Measurement of serum insulin and glucose. Insulin was measured by radioimmunoassay with an Insulin-MAIA Kit purchased from Serono Laboratories (Randolph, Mass.). The glucose oxidase method (Statzyme Glucose, Worthington) was used to measure glucose. The blood for these analyses was collected between 9:00 and 11:00 AM.

Data analysis. The data were evaluated statistically by the Student's *t* test; $P \leq 0.05$ was considered to be significant.

Results. *Influence of dietary lipids on tumor burden in diabetic and intact rats.* The effects of dietary lipids on mammary tumor and body weights for both diabetic and intact animals are summarized in Table II. In order to account for differences in dietary intake and its influence on body weight gain, tumor weight data are also presented as grams tumor/100 g body wt. In Experiment 1, tumor growth was studied in diabetic animals fed FF, HCTO, or HF diets. For comparative purposes, intact animals were fed either HF and FF diets (Experiment 2) or HF and HCTO diets (Experiment 3). The data presented here indicate that the relative effect of dietary lipids on mammary tumor growth differed in diabetic rats versus intact rats. In intact rats, grams tumor/100 g body wt was significantly greater in animals fed HF diets compared to animals fed either FF (Experiment 2) or HCTO (Experiment 3) diets. This relative increase in tumor weight per 100 g body wt in intact HF-fed rats was not observed in diabetic animals; in fact, tumor weight per 100 g body wt in diabetic rats fed HF diets was less than that seen in FF-fed

TABLE II. EFFECT OF DIET ON R3230AC TUMOR GROWTH IN DIABETIC AND INTACT FISCHER RATS

Experiment	Diet	No. of animals	Host[a] status	Tumor wt (g)	Body wt (g)	g tumor/100 g body wt
1	FF	18	Diabetic	3.58 ± 0.37[b]	84.3 ± 4.6	4.33 ± 0.47[d]
	HCTO	20	Diabetic	1.92 ± 0.37[d,f]	79.9 ± 2.9[c]	2.29 ± 0.35[c,f]
	HF	19	Diabetic	2.95 ± 0.31	91.5 ± 4.5	3.16 ± 0.25
2	FF	23	Intact	4.99 ± 0.28[e]	128.4 ± 1.9[e]	3.89 ± 0.31[d]
	HF	23	Intact	6.52 ± 0.41	137.8 ± 1.7	4.74 ± 0.31
3	HCTO	21	Intact	4.10 ± 0.35[e]	124.9 ± 1.9[e]	3.29 ± 0.29[e]
	HF	20	Intact	6.06 ± 0.47	132.6 ± 2.4	4.56 ± 0.34

[a] Animals were injected with streptozotocin, 60 mg/kg, ip.
[b] Values are means ± SE.
[c] Significantly less than HF ($P < 0.05$).
[d] Significantly greater or less than HF ($P < 0.025$).
[e] Significantly less than HF ($P < 0.01$).
[f] Significantly less than FF ($P < 0.01$).

rats. However, for both intact and diabetic rats, grams tumor/100 g body wt was significantly less in animals fed HCTO diets compared to animals fed HF diets.

A comparison of the tumor weight per 100 g body wt observed in intact and diabetic rats suggests that dietary lipids may have influenced the effect of diabetes on growth of this tumor. Actual tumor weights and body weights were lower in diabetic animals fed the same lipid diet as intact animals. When accounting for these differences in body weights, i.e., grams tumor/100 g body wt, it was observed that the relative tumor weights remained lower in diabetic animals fed either HF or HCTO diets compared to that in intact animals. However, for animals fed the FF diet, grams tumor/100 g body wt in diabetic rats were the same, if not greater, than those of intact rats. Therefore, animals ingesting diets devoid of lipids, i.e., FF diets, did not display slower tumor growth in the absence of endogenous insulin.

Insulin binding to tumor plasma membranes. The amount of specific ^{125}I-labeled insulin binding to tumor plasma membranes from diabetic animals fed FF, HCTO, or HF diets is presented in Table III. Binding of insulin was measured over a range of 2×10^{-10} to 10^{-8} *M*. The binding of insulin to membranes from HF-fed diabetic rats was significantly greater than binding to membranes of animals fed FF or HCTO diets ($P < 0.025$ and $P < 0.01$, respectively). Insulin binding to membranes from FF-fed rats appeared to be higher than binding to membranes from HCTO-fed rats, but this difference was not statistically significant. When the data were plotted according to Scatchard (20), typical curvilinear plots were obtained (not shown). Estimates of the binding parameters from these plots indicated that the differences in binding capacity (Table III) were due to alterations in the number of binding sites rather than to changes in the affinity of the receptor. The number of binding sites for the HF, FF, and HCTO membranes were estimated to be 6.2, 4.0, and 2.9 $\times 10^9$/100 μg protein, respectively, and the K_d was estimated to be 1.12, 0.88, and 0.88 n*M*, respectively.

Mammary tumor plasma membrane fatty acid composition. The effect of diet on the distribution profile of R3230AC plasma membrane fatty acids from diabetic rats is shown in Table IV; data are presented as percentage of total lipid. Comparison among the various diet groups indicated a number of differences. Membranes from rats fed HF diets had significantly higher proportions of the polyunsaturated fatty acids, C18:2 and C20:4, along with significantly lower percentages of the monounsaturated fatty acids, C18:1 and C21:1, than membranes from animals fed either FF or HCTO diets. It appeared that the membranes from the HF-fed rats contained somewhat less C16:1, but the difference was not statistically significant.

TABLE III. SPECIFIC INSULIN BINDING TO TUMOR PLASMA MEMBRANES

Insulin (*M*)	fmole insulin bound/100 μg protein		
	FF	HCTO	HF[a]
10^{-8}	20.0 ± 4.7 (7)[b]	11.7 ± 2.5 (5)	20.4 ± 1.7 (7)
4×10^{-9}	9.30 ± 1.5 (8)	8.31 ± 1.2 (2)	13.6 ± 1.5 (8)
8×10^{-10}	3.24 ± 0.54 (8)	2.28 ± 0.38 (7)	4.22 ± 0.32 (8)
2×10^{-10}	1.09 ± 0.18 (8)	0.88 ± 0.17 (6)	1.57 ± 0.14 (8)

[a] Statistical significance between the three diet groups was assessed at each insulin concentration by the Student *t* test. These *P* values were combined according to Fisher (29) and an overall comparison between the diet groups was made. Binding to HF membranes was significantly greater than binding to FF ($P < 0.025$) and HCTO ($P < 0.01$) membranes.

[b] Data are presented as means ± SE and numbers in parentheses represent the number of different membrane samples assayed.

Interestingly, C18:2 was present only in membranes from HF-fed rats whereas, C21:1 was present only in membranes from FF- or HCTO-fed rats. The effect of dietary lipids on the plasma membrane fatty acid profile in diabetic rats was similar to that reported previously (11) in intact rats, except for the proportion of C14:0. The percentage of this saturated fatty acid in membranes from intact rats ranged from 4 to 8% compared to the range of 0.47 to 1.32% found in membranes from diabetic rats. It should be noted that, in our earlier report (11), identification of C21:1 in membranes from FF- and HCTO-fed rats had not been firmly established. Reexamination of the gas–liquid chromatography tracings indicated that C21:1 was present in membranes of intact animals fed FF and HCTO diets, and was absent from intact animals fed HF diets.

Serum insulin and glucose levels. Serum insulin and glucose levels were determined in order to assess the severity of the diabetes induced in the animals (Table V). The serum glucose levels were markedly elevated in all animals studied. Average glucose levels of 615, 613, and 850 mg/dl, obtained for the HCTO-, HF-, and FF-fed animals, respectively, were markedly higher than glucose levels for intact animals; 136 ± 5 mg/dl for FF-fed rats and 139 ± 7 mg/dl for HF-fed rats. Diabetic animals fed the FF diets had significantly higher serum glucose levels than those fed the HF and HCTO diets, which we attribute to the greater amounts of carbohydrate in the FF diet. Intact animals, however, maintained better regulation of serum glucose levels, regardless of their dietary lipid intake. The serum insulin levels of the diabetic animals, 10.2, 13.3, and 12.2 μunit/ml obtained for the FF-, HCTO-, and HF-fed rats, respectively, were significantly lower than those obtained for intact animals (approximately 30–40 μunit/ml for HF- and HCTO-fed rats and approximately 50 μunit/ml for FF-fed rats). The higher serum insulin levels observed

TABLE IV. FATTY ACID COMPOSITION OF TUMOR PLASMA MEMBRANES FROM DIABETIC RATS[a]

Diet	C14:0	C16:1	C18:1	C18:2	C20:4	C21:1
HF	0.70 ± 0.70[b]	3.73 ± 1.9	17.2 ± 1.8	1.67 ± 0.35	23.0 ± 2.7	0 ± 0
HCTO	1.32 ± 1.32	5.60 ± 1.6	27.0 ± 2.2[c]	0 ± 0[c]	5.51 ± 1.29[c]	4.43 ± 1.72[d]
FF	0.47 ± 0.47	7.06 ± 1.3	24.5 ± 2.3[d]	0.16 ± 0.16[c]	10.7 ± 1.5[c]	2.53 ± 0.85[c]

[a] Relative percentages of only those fatty acids that show differences in response to altered diet are presented. Ranges for other fatty acids are as follows: C16:0, 24.7–29.1%; C18:0, 16.1–18.5%; C18:3, 0.26–1.38%.

[b] Values are means ± SE from three experiments.

[c] Significantly greater or less than HF ($P < 0.01$).

[d] Significantly greater than HF ($P < 0.05$).

TABLE V. EFFECT OF DIET ON SERUM INSULIN AND GLUCOSE LEVELS OF DIABETIC RATS

Diet	Serum glucose (mg/dl)	Serum insulin (μunit/ml)
FF	850 ± 48 (17)[a,b]	10.2 ± 0.7 (10)[c]
HCTO	615 ± 30 (17)	13.3 ± 0.8 (10)
HF	613 ± 35 (19)	12.2 ± 1.1 (10)

[a] Data are presented as means ± SE and numbers in parentheses represent the number of samples assayed.
[b] Significantly greater ($P < 0.001$) than HCTO or HF.
[c] Significantly less ($P < 0.01$) than HCTO.

in intact animals fed FF diets versus HCTO or HF diets were not seen in animals made diabetic by streptozotocin treatment.

Discussion. The data presented here suggest that (a) the insulin status of the host is an important factor in the ability of dietary lipids to alter growth of the R3230AC mammary tumor; (b) dietary lipids may influence the effect of diabetes on tumor growth; and (c) insulin binding to, and fatty acid composition of, the tumor plasma membranes were similar for both diabetic and intact animals. To assess the role of insulin and/or insulin binding capacity in dietary lipid alteration of tumor growth, the R3230AC mammary tumor was examined in both diabetic and intact rats. This transplantable tumor was selected because of its ability to grow in diabetic hosts (12, 13) and to demonstrate increased growth in intact animals fed a high polyunsaturated fat diet relative to fat-free or saturated fat diets (3, 11). In diabetic rats, however, growth of the R3230AC tumor was not found to be increased in animals fed HF vs FF diets. Previously, we postulated a role for insulin based on changes in tumor membrane insulin binding capacity that paralleled dietary lipid-induced alterations of tumor growth (11). We report here that insulin binding capacity of the plasma membranes, as well as their fatty acid composition, responded similarly to alterations in the dietary lipid for both diabetic and intact animals. We therefore suggest that the more rapid tumor growth seen in HF-fed intact rats was due, at least in part, to two components: insulin binding capacity and circulating insulin levels. In the data presented here, although insulin binding capacity was higher in HF-fed rats, the low levels of plasma insulin resulting from streptozotocin-induced diabetes may have precluded the enhancement of tumor growth. These results leave unanswered the question of whether variations in serum insulin levels, in response to dietary lipid manipulations, may lead directly to changes in tumor growth in the intact animal. However, in our earlier report, we found that altered serum insulin levels alone were not sufficient to alter growth of the tumor (11).

Two questions remaining to be resolved are (a) what factors are responsible for the greater tumor weights in the diabetic animals fed a FF diet vs a HF diet and (b) why, in diabetic animals, rats fed HCTO diets still experience less tumor growth compared to diabetic rats fed HF diets?

One possible explanation for the more rapid tumor growth in FF-fed rats is the presence of higher carbohydrate levels in the FF diet. From previous studies, it was found that noncarrier-mediated glucose entry into dissociated R3230AC cells represented approximately 10% of the glucose uptake, when studied at 2 mM glucose in the medium (21). Serum glucose in diabetic FF-fed rats was about five to seven times higher than that found in intact rats, and it is possible that this excess level of glucose, far exceeding the K_m for the glucose carrier (5 to 10 mM), resulted in greater entry of glucose into the tumor resulting in enhanced tumor growth.

The slower tumor growth seen in diabetic rats fed HCTO vs HF diets was similar to that seen in intact rats. Abraham *et al.* (22) had previously observed slower growth of normal mammary glands and mammary tumors in intact mice fed diets containing saturated fats vs polyunsaturated fats. It appeared that the 20% polyunsaturated lipid containing diet did not provoke greater tumor growth in the diabetic animal, since animals fed the HF diet did not display greater tumor growth than those fed a FF diet. Therefore, we conclude that the reduced tumor weight seen in HCTO-fed vs HF-fed diabetic rats was not due simply to the absence of polyunsaturated lipids, but rather to an effect of high saturated fat by a mechanism independent of insulin status of the animal. Others have reported that diets high in stearic acid

can inhibit tumor growth. Bennett (23), feeding A/ST mice a diet containing 13% stearic acid (same level as in the HCTO diet used here), observed a delay in the development of spontaneous mammary adenocarcinomas compared to mice fed a laboratory chow diet. Tinsley *et al.* (24) noted that increasing levels of dietary stearic acid were associated with decreased incidence and increased latency of spontaneous mammary tumors in C3H mice. Addition of increasing levels of stearic acid to the medium of primary cultures of both mammary epithelial cells and DMBA-induced mammary tumors resulted in a progressive inhibition of growth (25). One possible explanation for these findings could be due to an effect of stearic acid on the desaturase–elongation system responsible for converting linoleic acid to arachidonic acid (23).

Regardless of the diet ingested, a reduction in tumor growth was seen in diabetic animals compared to intact animals. While this may appear to be in contrast with previous findings (12, 13), differences in the severity of the diabetes, as reflected by the higher blood glucose levels and significantly lower body weights reported here, may have been responsible for the slower growth by the R3230AC tumor. It should also be noted that the earlier studies were conducted in laboratory chow-fed rats compared to the semisynthetic diets used in the present study. Curiously, when consideration is given to grams tumor/100 g body wt, an approach that would account for differences in body weight, diabetic rats ingesting FF diets displayed equal, if not slightly greater, tumor growth than intact animals fed the same diet.

It cannot be stated with certainty that the findings reported here for the R3230AC tumor would also be observed for other experimental mammary tumors, since, to our knowledge, this has not been investigated directly. However, the presence of insulin receptors has been established for DMBA-induced mammary tumors in Sprague–Dawley rats (26); growth of a majority of these tumors is dependent on insulin (27, 28). Additionally, for both R3230AC and DMBA-induced tumors, a role for estrogens in the dietary lipid effects on tumor growth has been suggested (1, 11). It would be of considerable interest to ascertain whether dietary lipid alterations might influence insulin binding in these carcinogen-induced neoplasms.

Taken together, data presented here support the earlier proposal that insulin plays a role in the dietary lipid modification of growth of the R3230AC mammary tumor. Alteration in plasma fatty acid composition was independent of the insulin status of the host, as was the relative order of insulin binding; effects on both of these parameters could be attributed to the lipid composition of the diet. In the absence of endogenous insulin, however, the difference in rate of growth of the tumor observed for HF-fed intact rats was not manifested, suggesting that insulin played a role in enhancement (HF vs FF) or retardation (FF vs HF) of tumor growth.

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