[CANCER RESEARCH 50, 5710–5719, September 15, 1990]

Review

Analysis of Dietary Fat, Calories, Body Weight, and the Development of Mammary Tumors in Rats and Mice: A Review

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Abstract

We have extracted from the literature data from 100 animal experiments, involving 7838 rats and mice, which compared the effects of different levels of dietary fat and/or calorie intake on the development of mammary tumors. Both higher calorie intake ($P < 0.0001$) and higher fat intake ($P < 0.0001$) independently increased mammary tumor incidence in Sprague-Dawley rats and in mice, as judged from analyses combining *ad libitum* feeding experiments and restricted feeding experiments. The effect of fat was two thirds the magnitude of the calorie effect in both Sprague-Dawley rats and mice. In *ad libitum* feeding experiments, a modest but significant ($P < 0.0001$) average increase in body weight was found in animals fed high fat diets. However, these differences in body weight did not correspond to differences in mammary tumor incidence. The effect of log body weight on the log odds of tumor incidence was not significant ($P = 0.16$), while dietary fat intake significantly increased tumor incidence ($P < 0.0001$). The collection of animal experimental data supports the hypothesis that, in mammary tumor development, there is a specific enhancing effect of dietary fat, as well as a general enhancing effect of calories.

Introduction

Over the past 45 years a considerable effort has been made to elucidate, by means of laboratory experiments on female rodents, the effect of dietary fat on the development of mammary tumors. This work has been reviewed by several authors (1–16).

There is general agreement that an increased intake of fat leads to an increased incidence in the number of mammary tumors. However, the effects of different sources of fat are still a matter of investigation and there is evidence of differences among their effects (17). For example, fish oil containing long-chain $n-3$ polyunsaturated fatty acids has been reported to protect against tumor development (18–20).

Several investigators have discussed the effect of varying total fat intake and different levels of total energy intake (21–25). However, no clear conclusions are available on whether the effect of fat intake on tumor incidence is modified by the level of calorie intake.

Many of the experiments have purported to achieve isocaloric intake by animals fed high and low fat diets (9). However, some have questioned whether the feeding experiments were designed to be truly isocaloric and have suggested that the animals fed high fat diets may retain more energy than those fed "isocaloric" low fat diets (26, 27).

In an attempt to resolve this dispute, investigators have compared the body weights of the animals on the two types of diets. Lack of a difference in average body weights would support the contention that the diets were truly "isocaloric." However, in reviewing the evidence, two authors (9, 15) suggested that there were no important differences in average body weights, while other investigators report the opposite conclusion (28, 29).

A closely related issue has been the question of the relative effects of fat and calories on mammary tumor development. Some suggest that the effect of high fat diets is entirely due to the resultant increased caloric intake (29–32), while others maintain that the effect of fat is independent of the calorie effect (9, 10).

In this article we bring evidence from a comprehensive review and analysis of the published experimental animal data to bear on the unresolved issues outlined above. Our overview differs somewhat from others in that we have attempted, where possible, to quantify effects by combining data from the various experiments.

For this overview only those experiments which compare different levels of the same fat are considered. We also restrict the analysis to those experiments which compare diets fed either before or shortly after administration of a chemical carcinogen (where given) and continued until the end of the experiment. Groups of animals fed experimental diets which were terminated before the end of the experiment are not included in this review.

We will, therefore, not pursue questions relating to differences in the effect of different sources and types of fat, the role played by the essential fatty acids, whether fat acts as an initiator of the carcinogenic process, and the effect of the duration of the diet on the development of mammary tumors.

The experimental animal studies on dietary fat and mammary tumorigenesis included in this overview are of some considerable relevance to studies of diet and breast cancer in women. Generally, the evidence from animal studies presently carries a heavier burden than usual in the forming of hypotheses on the relationship of diet to cancer in humans. This is due to the lack of reliance which can be placed on results from traditional analytical epidemiology, the case-control and cohort studies (33–36). Without these, we are left only with international comparisons, migration studies, and secular trends of breast cancer mortality and incidence to throw light on this subject (16). Thus, in the absence of more definitive human evidence, the animal evidence assumes more importance.

Methods

Literature Search. We conducted a literature search using the MEDLINE system to identify articles describing experiments with mice or rats in which were reported the effects of different amounts or sources of dietary fat upon the yield of mammary tumors. We used the following key words in the literature search: EXPERIMENTAL MAMMARY NEOPLASMS, MICE or RATS, and DIETARY FATS. The search covered the years 1966 to 1987. We identified further articles through citation in those articles found by MEDLINE. In particular, the review article by Albanes (37) was useful in identifying experiments carried out in mice before 1966.

Received 4/5/89; accepted 6/14/90.

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We perused each article to determine whether (*a*) the experiment comprised at least two experimental groups of rodents fed diets consisting of different amounts or sources of fat; (*b*) the composition of the experimental diets was reported; and (*c*) the proportion of animals with mammary tumors within each group was reported. From the 95 articles which appeared to satisfy these criteria, one of us (M. M.) extracted 31 items of information for each group of animals, including the following: carcinogen, dose of carcinogen, species (rat or mouse), strain, whether experimental diets commenced before or after carcinogen administration, number of animals, source of fat in the diet, percentage of fat in diet by weight, whether diet was fed *ad libitum* or restricted, total calories consumed/animal/day, final body weight, and proportion of animals with at least one tumor. The other two authors (C. C. and L. S. F.) checked the extraction and coding of the information for each article. During the extraction process we rejected additional articles completely (or retained only partial data) for a variety of reasons, including the following. Groups of animals were excluded if they were the subject of an additional procedure which could reduce or increase the tumor yield. For example, one experiment (38) comprised two groups of animals given high and low fat diets together with an immunotherapeutic drug, and another two groups fed high and low fat diets without drug. We included the latter two groups in our database but not the groups receiving immunotherapy. We also rejected experiments where the experimental diets were initiated more than 5 weeks after the carcinogenic insult, those where the carcinogen was fed as part of the diet, and those in which the carcinogen was administered at an age greater than 60 days. At the end of this process we were left with data from 68 articles to be analyzed.

Description of the Database. The database comprises data from 114 animal experiments extracted from 68 articles (20–22, 24, 38–101). The total number of animal groups included is 376, and the total number of animals is 11,033.

For the analyses in this paper we created two files from the database: (*a*) sets of animal groups in the same experiment which were fed, *ad libitum*, diets containing different levels of the same fat (20–22, 40–54, 56–60, 62, 64–69, 71–83, 86–90, 94, 96, 99, 100) (file 1) and (*b*) sets of animal groups in the same experiment which were fed either *ad libitum* or calorie-restricted diets containing the same fat source (21, 22, 24, 43, 62, 63, 69, 78, 84, 85, 92, 93, 95, 98, 101) (file 2). The level of fat fed to these groups may or may not have differed.

Each *file*, therefore, consists of a number of *sets* of animal groups as defined above. For the rest of the paper, we use the words file and sets to have the specific meanings given above.

Table 1 shows information about files 1 and 2 and their combination. Most of the sets in file 1 comprise rats and in the majority the fat source was corn oil. File 2 comprises sets of rats and mice in roughly equal proportion.

The combined file comprises 100 of the 114 experiments, 275 of the 376 animal groups, and 7,838 of the 11,033 animals in the full database. Of the 212 groups in which tumors were induced by 7,12-dimethylbenz(α)anthracene or methylnitrosourea, 100 were fed the experimental diet before the carcinogenic induction and continued on the experimental diet throughout the experiment. The other 112 groups were fed the experimental diet commencing within 5 weeks after carcinogenic induction and continuing to the end of the experiment. Fifty-seven groups comprised mice bred to develop tumors spontaneously. In one experiment, involving six groups of rats, tumors were induced by implantation of estrone pellets.

Twenty-two different sources of fat were fed in these experiments. The most common source was corn oil (149 groups), followed by lard (28 groups) and coconut oil (10 groups). The levels of fat in the diets fed to the groups ranged from 0 to 46% fat by weight (0 to 66% calories from fat). "Low fat" experimental diets most often contained 5% fat by weight (11% calories from fat) and "high fat" diets most often contained 20% fat by weight (36% calories from fat). Fourteen groups of mice were fed commercial laboratory chows in which the composition of diets and sources of fat were not identified. Nevertheless, these groups were included since they constitute 30% of the experiments on the effects of caloric restriction in the mouse.

Caloric intake by the groups was, of course, dependent on the species. There were also differences among different strains of rats. However, caloric intake was not reported for 162 of the 275 groups. Missing caloric intake data were mostly confined to the rat experiments. The range of caloric intake for Sprague-Dawley rats fed *ad libitum* was 45–65 kcal/day in those groups reported, with an average of 52.5 kcal. In mice the range was 10–17 kcal/day, with an average of 12.5 kcal. Combining rat and mouse studies, in the 24 *ad libitum* feeding experiments in which caloric intake was reported, animal groups on high ($\geq$15% calories from fat) and low fat (<15% calories from fat) diets had very similar total caloric intakes. The mean caloric intake among the groups fed low fat diets was only 0.3 kcal (SE = 0.5) lower than among the groups fed high fat diets, a nonsignificant difference.

Body weights were not reported for 55 of the 275 groups and were missing most often in the *ad libitum* feeding experiments.

The presence of tumor incidence information was a condition for inclusion in the database. The median tumor incidence among the groups was 67%, with upper and lower quartiles at 40% and 83%.

Calorie-restricted diets were fed to 49 groups, with the remaining 226 being fed *ad libitum*. The level of restriction ranged from 10% (*i.e.*, animals ate 90% of the *ad libitum* diet) to 58%.

Statistical Methods. We analyzed the data to answer the following four questions. (*A*) Does body weight differ systematically according to the level of fat fed in *ad libitum* feeding experiments? (*B*) Do the differences in body weight in *ad libitum* feeding experiments relate to the degree of mammary tumor development? (*C*) What are the effects on mammary tumor development of increasing caloric intake and fat intake, respectively? (*D*) Does the magnitude of the fat effect depend upon the level of total caloric intake?

Since questions *A* and *B* involve comparisons only of animal groups fed *ad libitum*, we used data in file 1 but not file 2 to address them; however, questions *C* and *D*, which involve comparisons of animal groups fed calorie-restricted diets with those fed *ad libitum* as well as comparisons of groups fed high and low levels of fat, were addressed using data from files 1 and 2 combined.

To assess the overall results from the literature, we used a quantitative method known by some workers as meta-analysis (102). This method is being used extensively to assess the effects of new therapies which have been tested in many different clinical trials (103). The idea is to combine the results of these trials into a summary measure which describes the average effect of the therapy. Results are combined, not by pooling all the data into one large data set and comparing patients on the new therapy with those on standard therapy, but by restricting comparisons of patients to those within the same trial and then pooling the results of such comparisons.

The same concept is applied in this review. All comparisons are made among groups of animals which were included in the same experiment and maintained under the same conditions apart from the stated differences in the diet. These comparisons are then combined to give a summary of the overall effect of the change in diet.

We performed a meta-analysis to answer each of the four questions (A–D) stated at the start of this section. To find whether body weight changed with the level of fat (question A), we used the statistical model:

$$\log_e(BW_{ij}) = M + SET_i + \beta(PCF_{ij}) + \epsilon_{ij} \qquad (I)$$

where BW_{ij} is the average final body weight of the animals in the j^{th} group of the i^{th} set, M is the overall intercept, SET_i is an adjustment factor for the i^{th} set, PCF_{ij} is the percentage of calories from fat fed to the j^{th} group of the i^{th} set, and ϵ_{ij} is experimental variation, which is assumed to be normally distributed. The coefficient β reflects the relation between body weight and level of fat in the diet and the focus of the analysis is to estimate β and determine its significance.

We chose to use the logarithm of body weight in order to facilitate an analysis across species. Since rats are much heavier than mice, similar relative changes in the weight of these animals, when fed different diets, are *a priori* more plausible than similar absolute changes in weight. Using a logarithmic scale for body weight is equivalent to analyzing relative changes in weight.

We chose percentage of calories from fat in model I rather than number of fat calories for the same reason, that is, to allow an analysis across species. Experimental diets fed to mice and rats were comparable

Table 1 *Contents of files 1 and 2 extracted from the database*

	Isocaloric experiments, same fat source, different levels of fat (file 1)		Experiments with restricted diets, same fat source (file 2)		Files 1 and 2 combined[a]	
	Comparative sets	Animal groups	Comparative sets	Animal groups	Comparative sets	Animal groups
Total	94	210	21	77	110	275
Rats (Sprague-Dawley)	86 (63)	189 (138)	9 (7)	35 (26)	90	212 (193)
Mice	8	21	12	42	20	63
Experimental diets started before carcinogen	43	98	1	4	43	100
Experimental diets started after carcinogen	45	95	7	25	49	112
Not applicable	6	17	13	48	18	63
Corn oil	60	133	7	23	64	149
Lard	13	26	1	2	14	28
Coconut oil	4	8	1	2	5	10
Other fats[b]	17	43	12	50	27	88
Total calories						
Known	24	61	16	59	37	113
Unknown	70	149	5	18	73	162
Final body weight						
Known	68	159	19	73	82	218
Unknown	26	51	2	4	28	57
Duration of experiment (from carcinogen)						
12–20 weeks	25	56	3	9	27	62
21–40 weeks	56	118	5	20	58	131
40–126 weeks	9	25	13	48	21	71
Unknown	4	11	0	0	4	11

[a] Columns for file 1 and file 2 do not sum to column for combined files because 5 sets (12 animal groups) were common to both files.
[b] Including fish, sunflower, olive, soybean, palm, safflower, and cottonseed oil.

with respect to percentage of calories from fat, whereas rats consumed a far greater number of fat calories (as well as total calories) than mice.

The percentage of calories from fat was calculated directly from the percentage of fat by weight using the approximate formula

$$\%\ \text{calories from fat} = \frac{9 \times \%\ \text{fat by weight}}{4 + 0.05 \times \%\ \text{fat by weight}}$$

This formula is based on the Atwater values (104) and the assumption that the weight of the diet is the weight of the fat, carbohydrates, and proteins combined.

We used similar statistical models to address questions B, C, and D. To find whether final body weight differences related to differences in degree of tumor development (question B), we used the following model:

$$\log_e\left(\frac{p_{ij}}{1 - p_{ij}}\right) = M + SET_i + \beta_1 \log_e(BW_{ij}) + \beta_2 PCF_{ij} \qquad \text{(II)}$$

where p_{ij} is the proportion of animals in the j^{th} group of the i^{th} set which develop one or more mammary tumors during the experiment and $\log_e\left(\frac{p_{ij}}{1 - p_{ij}}\right)$ is the log odds of tumor incidence. Logarithm of body weight and percentage of calories from fat were chosen in model II for the same reasons as in model I. The coefficient β_2 reflects the effect of fat level on the log odds of tumor incidence in the absence of any change in body weight, whereas β_1 reflects the effect of body weight on the log odds of tumor incidence for a given level of fat in the diet. If the fat effect were explained by the difference in energy retained by animals on low and high fat diets, then, taking body weight as a measure of retained energy, one would expect to obtain from analysis, using the model above, a large positive value for β_1 and a small nonsignificant value of β_2.

The body weights of animals were not reported in a proportion of the experiments. The data from these experiments could not be used to examine questions A and B and were, therefore, excluded from analyses using models I and II.

Question C involves the caloric uptake as well as the level of dietary fat. We assess this question using the model:

$$\log_e\left(\frac{p_{ij}}{1 - p_{ij}}\right) = M + SET_i + \beta_1 TCAL_{ij} + \beta_2 FCAL_{ij} \qquad \text{(III)}$$

where $TCAL_{ij}$ is the average total kcal consumed/animal/day in the j^{th} group of the i^{th} set and $FCAL_{ij}$ is their average consumption of fat in kcal.

We chose the measure of fat intake in this model to be number of fat calories rather than percentage of calories from fat in order to allow separation of the calorie from the fat effect. The coefficient β_1 represents the effect on tumor incidence of raising total calorie intake by 1 kcal while maintaining the same fat intake, *i.e.*, increasing non-fat intake by 1 kcal. The coefficient β_2 represents the effect of raising fat intake by 1 kcal while maintaining the same total calorie intake by a corresponding reduction in non-fat calories, *i.e.*, the difference between the effect of eating 1 kcal of fat and 1 kcal of non-fat ingredients. The relative magnitudes of β_1 and β_2, therefore, provide a quantitative comparison of the calorie effect with the fat effect, while a significance test of β_2 evaluates the evidence for the hypothesis that there is no separate fat effect. Using percentage of calories from fat instead of number of fat calories in this model would be confusing, since β_1 would then represent the effect of raising total calorie intake by 1 kcal while maintaining the same percentage of calories from fat, *i.e.*, the effect of increasing intake by 1 kcal of a mixture of fat and non-fat ingredients in unspecified proportion.

Because we use number of fat calories and number of total calories in model III, an analysis across species is no longer sensible, for reasons given above in the discussion of models I and II, *i.e.*, because rats consumed a far greater number of fat and total calories than mice. Therefore, we conducted separate analyses of mice and rats. At the end of this section, we indicate precisely which data we have analyzed using each of the models described.

Question D was assessed by adding an extra term, β_3 ($TCAL_{ij} \times FCAL_{ij}$), to the right side of model III. The coefficient β_3 reflects the extent to which the fat effect changes with increasing total calorie intake. If the effect of fat were constant over the range of caloric intakes in the database, then the estimate of β_3 should be close to zero and statistically insignificant.

Unlike questions A and B, questions C and D do not involve animal body weight. We were, therefore, able to include in these analyses data from experiments for which body weights were not reported.

Total calorie intake was not reported in the majority of *ad libitum* feeding experiments nor in some restricted feeding experiments. We conducted two analyses of questions C and D. The first analysis included data only from experiments for which total caloric intake was recorded. The second analysis utilized also data from experiments with unreported caloric intake. For these experiments we estimated caloric intake in the following way. For groups of animals fed *ad libitum*, the caloric intake was estimated as the average reported caloric intake of groups of the same strain, regardless of level of fat in the diet. For groups fed restricted diets, an estimate of the caloric intake could be calculated from this average value using the percentage of caloric restriction, which was always reported. Fat calories were estimated by multiplying the estimated total caloric intake by the proportion of calories from fat.

This second analysis is justified on the grounds that, firstly, in *ad libitum* feeding experiments caloric intake appeared not to differ according to the level of fat in the diet (see "Description of the Database") and, secondly, within each strain of species the range of caloric intake in *ad libitum* feeding experiments was quite narrow. The merit of the second analysis is that it allows inclusion of considerably more data which bear on questions C and D.

We have mentioned above that we were concerned to choose, where possible, models which would plausibly carry the same values of the coefficients for different species. We wished to check whether indeed the coefficients were similar in different subgroups of experiments. Apart from species, two other possibly important factors were strain of rat and source of fat. We, therefore, conducted analyses within different subgroups. In this paper we present the results of two important subgroups: Sprague-Dawley rats (the strain most often studied) fed corn oil (the source of fat most often used) and mice, bred to develop spontaneous tumors, fed any source of fat. In Table 2 we tabulate the data sources for the analyses of each question.

The goodness-of-fit of models I–III was assessed by plotting the adjusted dependent variable against the independent variable in the model. A perfect fit to the model would be indicated by the points lying on a straight line through the origin.

The analysis was performed using the GLIM (Generalized Linear Interactive Modeling) package (105) on the National Institutes of Health DEC 10 computer.

Results

Question A

The question about whether body weight increases with fat level was addressed using the 68 sets of animals from file 1 for which final body weight was reported. A total of 159 groups of animals were included in these sets.

Fig. 1 displays the adjusted log body weight plotted against the percentage of calories from fat. It can be seen that body weight tends to be higher in groups fed high fat diets. The trend is apparently linear with percentage of calories from fat. In fact, of the 68 sets, in 49 the highest fat group had a greater average body weight, in 7 there was no difference, and in 12 the highest fat group weighed less on average than the lowest fat group.

Table 3 shows the results of fitting model I. The value of β

Table 2 *Data sources for the analyses of each question*

Question	File	Subgroup	Experiments excluded
A and B	1	1. Total file 2. SD[a] rats fed corn oil 3. Mice[b]	1. Body weight unreported
C and D	1 and 2 combined	1. SD rats fed corn oil 2. Mice[b]	1. Calorie intake unreported 2. No exclusions

[a] Sprague-Dawley.
[b] Bred to develop spontaneous tumors.

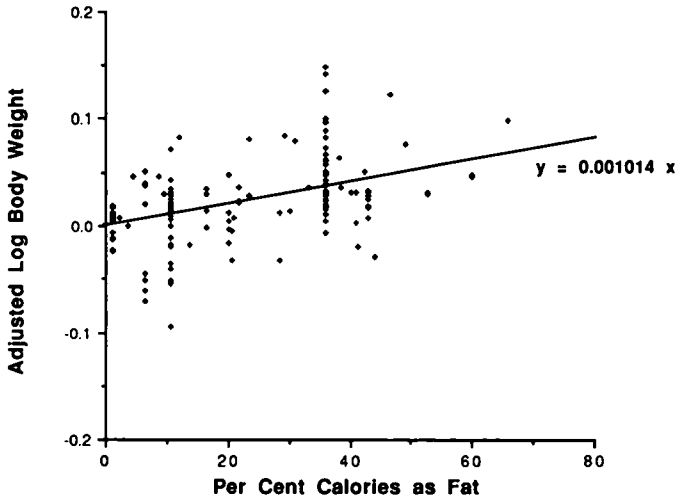

Fig. 1. Log body weight, adjusted for experiment, plotted against percentage of calories as fat.

Table 3 *Results of fitting model I to the data*

Data	No. of groups	Estimation of β[a]	SE	z[b]	P value[c]
All sets in file 1[d]	159	0.00101	0.000240	4.23	<0.0001
Sprague-Dawley rats fed corn oil[d]	74	0.00072	0.000184	3.92	<0.0001
Mice bred for spontaneous tumors[d]	15	0.00098	0.00128	0.76	0.44

[a] This is the estimated increase in $\log_e(BW)$ resulting from an increase of 1% fat from calories in an isocaloric experiment. The corresponding proportional increase in body weight is given by $[\exp(\beta) - 1] \times 100\% = 0.10\%$ for all sets in file 1. The proportional increase in body weight resulting from an increase of 10% fat from calories in an isocaloric experiment is estimated as $[\exp(10\beta) - 1] \times 100\% = 1.01\%$ for all sets in file 1 (see text).
[b] z = estimate/SE.
[c] Assuming z is a standard normal deviate (Wald test).
[d] Excluding sets where body weight is unknown.

is positive and statistically significant, indicating that the overall evidence is strongly in favor of an increased body weight in animals fed *ad libitum* diets with a high fat content. The magnitude of the increase, however, appears modest. Based on the estimate of 0.001 for β, one may calculate that the body weight is increased on average by an extra 1% for every added 10% of calories from fat. Thus, if Sprague-Dawley rats fed a diet containing 10% calories from fat weighed 300 g at the end of the experiment, then we might expect an equivalent group fed 20% calories from fat in an *ad libitum* feeding experiment to weight 303 g. This 1% increase is an average figure and the actual increase may depend on other factors such as the strain or species of animal and the source of dietary fat. For this reason, we estimated β within subgroups which were more homogeneous. The results for two important subgroups, Sprague-Dawley rats (the strain of rat most often studied) fed corn oil (the source of fat most often studied) and mice fed any source of fat, are shown in Table 3. The estimates of β are similar to that obtained for the whole file, as are the estimates of β for other subgroups not reported here.

Question B. Having established that there is a greater body weight in animals on a high fat diet fed *ad libitum*, we proceed to the second question, namely, do the changes in body weight observed in *ad libitum* feeding experiments explain the increases in mammary tumor incidence reported?

To examine this question we analyzed all sets in file 1 for which the body weight is known and also the two subgroups mentioned above: Sprague-Dawley rats fed corn oil (32 sets comprising 74 animal groups) and mice fed any source of fat (5 sets comprising 15 animal groups). Table 4 shows the result

Table 4 *Results of fitting model II to the data*

Data	Log body weight (g)				Calories from fat (%)			
	Estimate of β_1	SE	z^a	P^b	Estimate of β_2	SE	z^a	P^b
All sets in file 1[c]	1.5	1.2	1.27	0.20	0.035	0.0028	12.5	<0.0001
Sprague-Dawley rats fed corn oil[c]	−3.8	2.7	−1.41	0.16	0.043	0.0041	10.5	<0.0001
Mice bred for spontaneous tumors[c]	1.7	1.8	0.93	0.35	0.031	0.0081	3.8	0.0001

[a] z = estimate/SE.
[b] Assuming z is a standard normal deviate (Wald test).
[c] Excluding sets where body weight is unknown.

of fitting statistical model II to these data. For both the full data set and the two subgroups, the estimated coefficients for the fat effect (β_1) are positive, highly significant, and of similar magnitude. For example, one may calculate from the estimate of β_2, 0.043, for the Sprague-Dawley rats fed corn oil that an extra 10% calories from fat in a diet fed *ad libitum* would raise tumor incidence from a baseline 50% to 61%.

The estimated coefficient for body weight is positive for the full data set and negative in the rat subgroup but not statistically significant in either case. The nonsignificance of the coefficient suggests that the mostly small differences in final body weight which are seen in these *ad libitum* experiments simply have little or nothing to do with the increase in mammary tumor incidence observed.

As an example of the goodness of fit of the model, Fig. 2 displays the adjusted log odds of tumor incidence plotted against percentage of calories from fat for the subgroup of Sprague-Dawley rats fed corn oil in the analysis just described. It can be seen that the linear model fits quite well, aside from two groups fed the highest levels of fat but with apparently low tumor incidence. These groups were from two atypical studies (77, 81) in which feeding of the experimental diets commenced just less than 5 weeks after the administration of carcinogen, the experimental diets were fed for only 8 weeks, and one diet contained more than 50% calories from fat.

As mentioned in "Methods," there was little difference between total caloric intake of animals fed high and low fat diets in *ad libitum* feeding experiments. Thus differences in total caloric intake do not explain the differences in average body weight.

Question C. The preceding analysis established the effect of fat on mammary tumor development in *ad libitum* feeding experiments. However, from these experiments, nothing can be said about the effect of increased energy intake. In question C we asked what are the relative effects of energy intake and fat intake and turned to experiments with calorie-restricted diets; we analyzed data from the combined files 1 and 2.

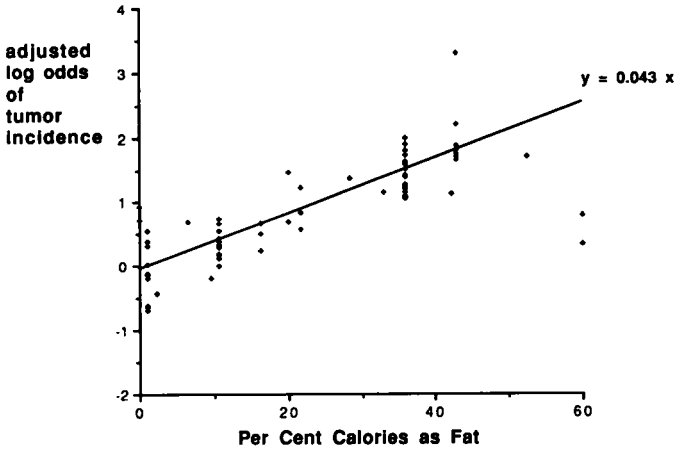

Fig. 2. Log odds of tumor incidence, adjusted for experiment and log body weight, plotted against percentage of calories as fat.

To address the question we used model III. This model relates tumor incidence to total calorie intake and fat calorie intake. Preliminary analysis showed the magnitude of the effect of reducing calorie intake (either total or from fat) by 1 kcal to be quite different among mice and rats. We, therefore, applied model III not to the total combined files 1 and 2 but, separately, to the two subgroups considered earlier in relation to questions A and B: Sprague-Dawley rats fed corn oil and mice fed any source of fat. We could not restrict our analysis to sets of mice fed a particular source of fat, since 14 groups were fed a commercial laboratory chow diet. The other sources of fat fed to these groups of mice were hydrogenated cottonseed oil (22 groups), cottonseed oil plus soybean oil (9 groups), corn oil (4 groups), Crisco (4 groups), lard (2 groups), and soybean oil (2 groups).

The subfile of Sprague-Dawley rats fed corn oil comprised 104 animal groups in 43 comparative sets, including experiments for which total caloric intake was not recorded. Results of fitting model III to these data are shown in the first row of Table 5. These results show that both total calories and fat calories have effects on tumor incidence which are highly significant, even when one effect is adjusted for the other. The effect on the log odds of tumor incidence of raising total calories by 1 kcal while keeping fat calories fixed is estimated to be approximately 1.5 times the effect of raising fat calories by 1 kcal while keeping total calories fixed. Raising total calories by 1 kcal while keeping fat calories fixed would increase the tumor incidence from a baseline of 50% to 53.1%, as calculated from the estimate of β_1. A similar calculation based on the estimate of β_2 shows that raising fat calories by 1 kcal while keeping total calories fixed would raise the tumor incidence from a baseline of 50% to 52.0%. The second row of Table 5 shows the results of analyzing the same subfile but excluding experiments in which total caloric intake was not recorded. Excluding these experiments has little effect on the results.

The mouse subfile comprised 57 animal groups in 17 comparative sets, including experiments with total caloric intake not recorded. The results of fitting model III to these data are shown in the third row of Table 5. Like the analysis of Sprague-Dawley rats fed corn oil, the results show highly significant effects for total calories and fat calories, and again the total calorie effect is approximately 1.5 times the fat calorie effect. Excluding experiments in which total caloric intake was not recorded does not materially change the results (fourth row of Table 5). The magnitude of these effects in the mice is approximately 5 times that of the effects observed in the Sprague-Dawley rats. Sprague-Dawley rats fed *ad libitum* eat about 4–5 times the calories consumed by mice. We would, therefore, expect the effect of an extra 1 kcal to be correspondingly greater in the mouse than the Sprague-Dawley rat.

Fig. 3, *a* and *b*, shows the adjusted log odds of tumor incidence plotted against total calories and fat calories, respec-

Table 5 *Results of fitting model III to the data*

Data	No. of groups	Total calories (kcal)				Fat calories (kcal)			
		Estimate of β_1	SE	z^a	P^b	Estimate of β_2	SE	z^a	P^b
Sprague-Dawley rats fed corn oil									
Including kcal unknown	104	0.125	0.018	6.9	<0.0001	0.081	0.0060	13.5	<0.0001
Excluding kcal unknown	25	0.126	0.023	5.5	<0.0001	0.060	0.0112	5.3	<0.0001
Mice bred for spontaneous tumors									
Including kcal unknown	57	0.627	0.049	12.9	<0.0001	0.402	0.046	8.8	<0.0001
Excluding kcal unknown	51	0.619	0.050	12.4	<0.0001	0.490	0.060	8.2	<0.0001

[a] z = estimate/SE.
[b] Assuming z is a standard normal deviate (Wald test).

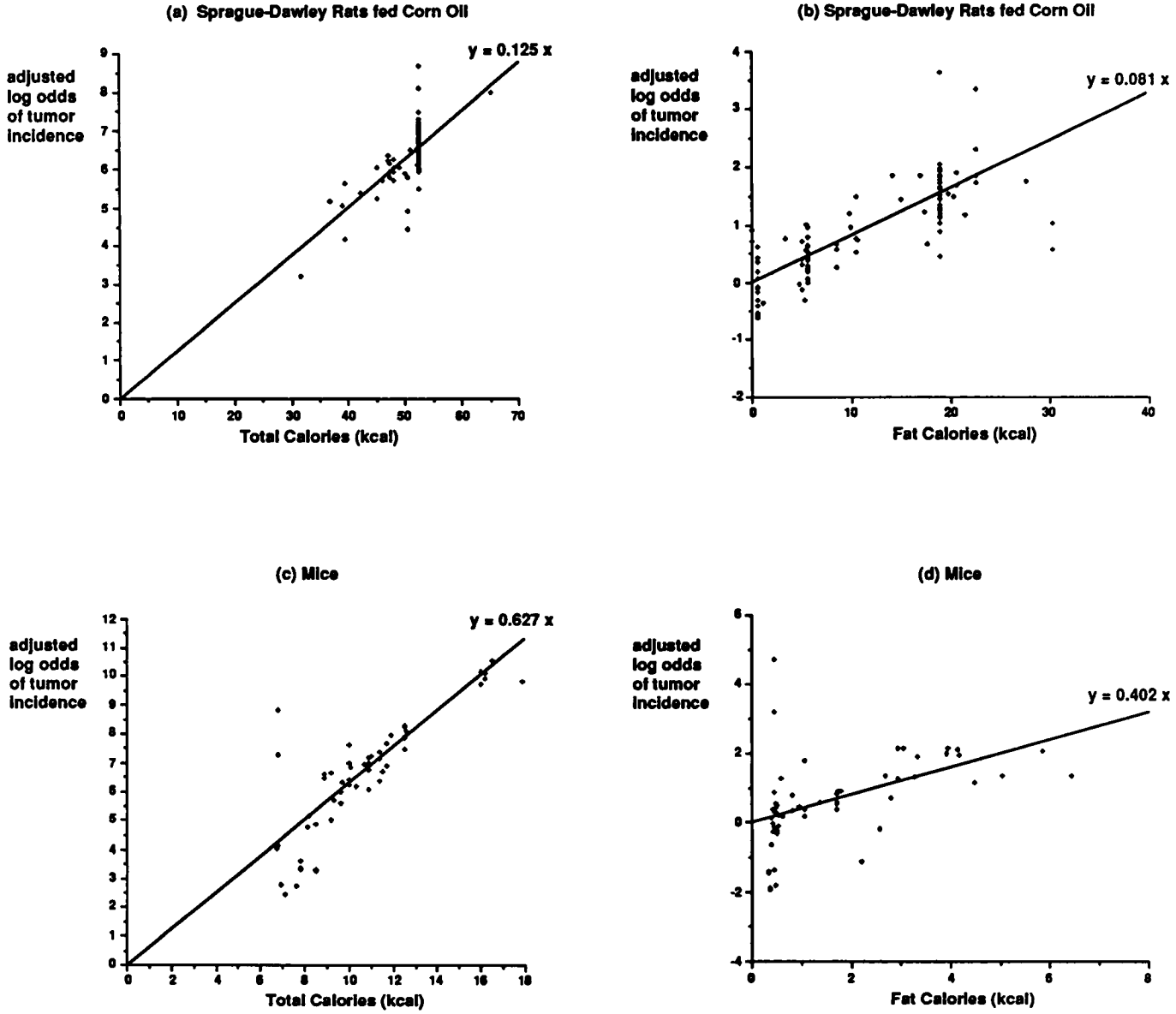

Fig. 3. Adjusted log odds of tumor incidence plotted against total calories for experiments with Sprague-Dawley rats fed corn oil (*a*), fat calories for experiments with Sprague-Dawley rats fed corn oil (*b*), total calories for experiments with mice bred for spontaneous tumors (*c*), or fat calories for experiments with mice bred for spontaneous tumors (*d*).

tively, for Sprague-Dawley rats, and Fig. 3, *c* and *d*, shows the same for the mice. The plots for Sprague-Dawley rats show no clear departures from linearity except the two outliers in Fig. 3*b*, which are the same groups noted previously in Fig. 2. Fig. 3*c* shows a relationship between log odds of tumor incidence and energy intake which is close to linear. Fig. 3*d* shows widely scattered points at low levels of fat intake. These are groups with very low levels of tumor incidence which do not strongly influence the overall regression line. For the central band of points there is a clear trend with fat intake, with a suggestion of a quadratic component. Overall, the linear model appears a reasonable description of the relationship between log odds of tumor incidence and intake of total calories or fat calories.

Question D. The question of whether the fat effect is dependent on the level of calorie intake was addressed by fitting model III with an extra term of interaction between total calories and fat calories. We performed this analysis for the same subfiles used to address question C. The analysis for Sprague-Dawley

rats yields no strong evidence for the presence of such an interaction ($z = 1.00$, $P = 0.32$). Thus there is no indication that the fat effect varies with the energy intake. For mice, however, the coefficient for the interaction term is negative and statistically significant ($z = -2.98$, $P = 0.003$). This indicates a tendency for the fat effect to be larger at lower levels of energy intake. The magnitude of the interaction, however, has only a slight effect on the variation in fat effect over the usual range of calorie intake in mice. For example, considering a mouse restricted to 8 kcal/day, the effect of increasing fat consumption by 1 kcal while keeping total caloric intake constant would be to increase a tumor incidence of 50% to 65.5%. In contrast, the effect of increasing fat intake by 1 kcal in a mouse consuming 12 kcals *ad libitum* would be to increase a tumor incidence of 50% to 59.9%. Thus the effect of fat is clearly to promote mammary tumor development, both at levels of energy usually consumed by mice on *ad libitum* diets and at restricted levels of energy intake.

Discussion

When many studies of the same basic question have been undertaken, combining the results often leads to a clearer overall message than examination of individual studies. There are two reasons for this. Firstly, each individual study is based on relatively small sample sizes, so that random variation can play a greater part in distorting the observed effect. However, in combination the random effects tend to cancel each other and thus have less influence. Secondly, experimental conditions vary from study to study. Combining results over many experiments enables one to investigate an effect over a broad range of settings and gives greater confidence in the generality of the conclusion.

However, quantitative review of the literature is subject to difficulties and these have been discussed extensively (102), particularly with regard to overviews of clinical trials (106). Major concerns are the methods of ascertainment, the selection of studies for inclusion in the overview, and the accuracy of the data. For clinical trials, overviews which include only published articles are likely to be affected by publication bias: trials which do not show treatment differences are less likely to be published, so the review may overestimate the treatment difference. This effect is less likely to be a problem in our review, since there has long been controversy over the effect of fat on mammary tumorigenesis, and both positive and negative results have been of interest. We have tried to select the data for inclusion in our review in an objective and unbiased manner. As detailed in "Methods," decisions to include or exclude articles were based on the design of the experiment and the adequacy of the reported data, rather than the results.

The main items of information employed in our analysis are body weight, tumor incidence, level and source of fat in the diet, and total caloric intake. Tumor incidence was nearly always reported as that based on autopsy findings, not on palpation of the live animal. Aside from the early mouse experiments, we included only articles where the level of fat in the diet and the source of fat were clearly specified.

In the majority of *ad libitum* feeding experiments, total caloric intake was not available. However, our two analyses, one excluding those experiments which did not report caloric intake and the other including these experiments, led to the same conclusions.

Our calculation of percentage of calories from fat assumes that the weight of dietary ingredients other than fat, carbohydrate, and protein was negligible. In fact, these ingredients accounted for 5–15% of the total weight of the diet in the experiments we considered. An analysis of question C, using a more exact calculation which accounted for the weight of these ingredients, led to very similar estimates of the fat and caloric effects and to the same conclusions as the analysis which we have presented.

Body weight measurements have not been reported in a standard manner. The majority of investigators reported the body weight at autopsy, for those animals sacrificed at the end of the experiment. Others reported the body weight averaged over all animals, regardless of when they were sacrificed. The latter method may be biased towards finding lower body weights in the animals on high fat diets, since they develop tumors earlier and are, therefore, sacrificed earlier in the experiment. Since animals continue to gain weight throughout the experiment, those sacrificed earlier will tend to be lighter. Nearly all investigators reported the body weights of animals including the weight of any tumors which the animals might bear at the time of autopsy. Only one article (91) gave the average weight of the carcasses of the animals from which the tumors had been removed. Thus animal groups on high fat diets which develop more tumors might be expected to weight more because of their extra tumor burden. Assuming the average weight of tumor to be about 3 g (56) and supposing a high fat group to have 30% more animals with tumors than a low fat group, we would perhaps expect the resulting difference in average total body weight to be 0.3×3 g, *i.e.*, about 1 g. As shown in the example in "Results" pertaining to question A, Sprague-Dawley rats fed 10% more of their calories from fat can be expected to be heavier by an average of 3 g. The extra weight of tumors may explain some but probably not all of this difference in body weight.

In a previous review of a dozen published articles, Welsch (15) concluded that there was no significant increase in the average weight gain of animals on a high fat diet, compared with that of animals on a low fat but isocaloric diet. However, Pariza (29) and Jacobson *et al.* (28) more recently reported studies in which the animals fed high fat diets *ad libitum* weighed more than those fed a diet low in fat. Our overview shows that average weights, as reported, tend to be higher in animals on *ad libitum* high fat diets, compared to those on *ad libitum* low fat diets. However, the overall difference is small.

The observation of a slightly greater body weight in groups of animals fed an *ad libitum* diet high in fat may be due to such a diet providing more usable energy (27), but this conclusion is open to doubts stemming from the variable methods used to measure body weight, as mentioned above. Let us suppose, however, that the observed increases in body weight are indeed real gains in weight resulting from the greater amount of energy retained from the diet. Our analysis using model II has shown that the changes in body weight do not explain the increased tumor incidence in the high fat groups.

This conclusion is reinforced by evidence from four restricted diet experiments in Sprague-Dawley rats in which one group of animals was fed 50 kcal/day and the other 35 kcal/day of the same diet (22). From the average difference in body weights in this study, one may estimate that an increase in log body weight of 0.01 would be produced by increasing the intake by 0.83 kcal, *i.e.*, a little under 1 kcal/day. Since this is the order of body weight increase in animals fed *ad libitum* a diet containing 10% more calories from fat, we may estimate that such a diet yields a little under 1 kcal extra energy/day to each rat. From the results of model III for Sprague-Dawley rats, a group with

10% more calories from fat would be expected to have their tumor incidence raised from 50% to 52.6% due to the increased energy intake. However, the fat effect estimated from model II leads to an expected incidence of 60% in such a group. Thus, the overall increase in body weight in groups fed high fat diets is too small to account for the magnitudes of the increases in tumor incidence which have been observed.

Silverstone and Tannenbaum (88) presented a detailed critique of the hypothesis postulated by Boutwell *et al.* (107) that the fat effect is due to the increasing efficiency of utilization of diets high in fat. Using the work of Forbes *et al.* (108–111), Silverstone and Tannenbaum show that mice fed a diet containing 27% fat by weight may retain 0.3 kcal/day more energy than those fed a diet containing 2% fat by weight. They conclude, like ourselves, that this increase in energy is not sufficient to explain the increase in tumor incidence observed.

Boutwell (32; page 94) states unequivocally: "Cancer incidence in specific experimental models is not dependent on the percentage of fat in the diet nor on the quantity of fat consumed. Rather, the level of caloric intake *versus* caloric expenditure determines cancer incidence." The results of this review clearly contradict this conclusion in the case of mammary tumorigenesis. When data from calorie-restricted and *ad libitum* feeding experiments are combined, our results using model III show that tumor incidence is modified both by fat consumption and by total energy consumption. Our analyses indicate that the increase in mammary tumor incidence which results from consuming more fat is higher than the increase in incidence which would result from consuming the same amount of extra calories as non-fatty foods.

As strong supporting evidence of his statement, Boutwell cites the quantitative review conducted by Albanes (37). We have reanalyzed the mouse data reported in Albanes' review, using the methodology described in this paper and in particular employing model III. Our results for the experiments involving mammary tumors were very similar to those presented here, with highly significant effects for both total energy intake and fat intake. In contrast, no separate effect for fat intake was found in the group of experiments involving skin tumors. It, therefore, seems mistaken to draw conclusions about the fat hypothesis from Albanes' combined analysis. A criticism of Albanes' analysis is that it violates the "meta-analysis rule" that one should compare groups only within experiments.

Another result, which is commonly cited as evidence against the fat hypothesis, is the experiment of Boissoneault *et al.* (43). This experiment was conducted in F344 rats, a strain of rat which we did not include in our main analyses. We have separately analyzed the data from this experiment employing model III. The estimate of the coefficient β_2 for the fat effect is 0.124 (SE = 0.056), a little larger than the estimate of 0.081 derived from our analysis of Sprague-Dawley rat experiments. This result occurs because the group of rats fed a low fat diet *ad libitum* consumed more calories than the group fed a high fat diet *ad libitum*, yet developed fewer tumors. A third group of rats fed a restricted high fat diet had no control group fed a restricted low fat diet and, therefore, yields little information about the fat effect. Thus, the results of the Boissoneault experiment appear to support the fat hypothesis.

We were able to investigate whether the effect of fat on tumor incidence is modified by the level of calorie intake. Our review of experiments with Sprague-Dawley rats fed corn oil shows no evidence of a reduced effect of fat in animals on restricted feeding. However, it appears that a diet with increased fat intake may be more effective in stimulating tumor growth under conditions of calorie restriction, compared to *ad libitum* feeding, in the spontaneous mouse tumor model. Since the same effect is not seen in rats, the phenomenon may be specific to certain species and its relevance to human cancer is, therefore, uncertain.

The results from this review are important because much of the known evidence on the relationship of dietary fat and breast cancer is confusing, particularly the results of case-control and cohort studies (16). In the past, doubt as to the strength of the cumulative evidence from the mouse and rat models has played a part in deterring the establishment of studies to evaluate the effect of a low fat diet intervention on breast cancer incidence in women (32). We have shown in the two animal models most commonly studied, 7,12-dimethylbenz(α)anthracene-induced mammary tumors in the Sprague-Dawley rat and spontaneous mammary tumors in inbred strains of mice, that increased total fat intake clearly enhances the development of these tumors. Moreover, the greater incidence of tumors in animals with increased total fat intake occurs even when total energy intake is kept constant. Thus, if animals consume a diet which is higher in fat and in total energy, then both factors will act separately to increase the tumor incidence. Conversely, diets which are lower in fat and in total energy will decrease tumor incidence by reducing both the total fat intake and the energy intake. This suggests that future research studies relating dietary modification to human breast cancer should include reduction of both total fat and total calories. The rationale for such studies is supported by the body of animal experimental data available to us today.[2]

Acknowledgments

We thank Dr. D. Byar, who suggested the idea of this project, provided advice throughout, and supplied references. Dr. D. Albanes generously allowed us access to his database in the pilot phase of the project. J. Gaegler gave excellent secretarial support, and Tom Riley helped with the data preparation and computing.

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[2] Since conducting the analysis an additional five articles (28, 112–115) have been found which could have been added to the database. The article by Jacobson *et al.* (28) appeared after the start of our analysis. Three other articles (112–114) were primarily concerned with an issue other than dietary fat but included groups of animals fed high fat and low fat diets. The remaining article (115) is an expanded report of an experiment (82) already included in our database. Extra information in this report would allow inclusion of an additional three animal groups. These articles all involved comparisons of high fat with "isocaloric" low fat diets. The results of the experiments are qualitatively the same as the overall conclusions of our report. The groups on the high fat diet all had a higher incidence of tumors than the corresponding groups on the low fat diet and were heavier at the end of the experiment. Thus, overall conclusions from our analysis would not be altered by the inclusion of these extra data.

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