Effect of Different Types and Amounts of Fat on the Development of Mammary Tumors in Rodents: A Review

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ABSTRACT

We performed a meta-analysis on data extracted from 97 reports of experiments, involving a total of 12,803 mice or rats, studying the effect on mammary tumor incidence of different types of dietary fatty acids. Fatty acids were categorized into saturated, monounsaturated, n-6 polyunsaturated, and n-3 polyunsaturated. We modeled the relation between tumor incidence and percentage of total calories from these fatty acids using conditional logistic regression and allowing for varying effects between experiments, and for each fatty acid we estimated the effect of substituting the fatty acid calories for nonfat calories. Our results show that n-6 polyunsaturated fatty acids (PUFAs) have a strong tumor-enhancing effect and that saturated fats have a weaker tumor-enhancing effect. The n-3 PUFAs have a small protective effect that is not statistically significant. There is no significant effect of monounsaturated fats. n-6 PUFAs have a stronger tumor-enhancing effect at levels under 4% of total calories, but an effect is still present at intake levels greater than 4% of calories. In addition, when the intake of n-6 PUFAs is at least 4% of calories, the n-6 PUFAs effect remains stronger than the saturated fat effect.

INTRODUCTION

In a quantitative review of the literature, Freedman et al. (1) reported that dietary fats enhance mammary tumors in mice and rats and that this effect is not simply the result of increased energy intake from the fat. Freedman et al. (1) studied the tumor-enhancing effect on mice fed different types of fat and on Sprague-Dawley rats fed corn oil, a fat that is mostly composed of linoleic acid, an n-6 PUFAs. 2 Reviews of animal experiments since that time (2–7), as well as further work by Freedman and colleagues (8, 9), have stressed the differential effects of different fatty acids. Since the 1990 report by Freedman et al. (1), we have updated the database and quantified the intakes of different types of fatty acids. In this paper, we analyze data from the updated database to address some proposed hypotheses regarding the differential effects of fatty acids.

We categorize the type of dietary fat into saturated, monounsaturated, n-3 polyunsaturated, and n-6 polyunsaturated fatty acids and model their respective effects on mammary tumor incidence. We then address three previously proposed hypotheses (10–14):

(a) We investigate whether there is a saturation point for n-6 PUFAs, i.e., an intake level of n-6 PUFAs beyond which no further tumor promotion occurs. Because most n-6 PUFAs in the diet are EFAs, it is hypothesized that once the requirement for EFAs is met, additional intake of n-6 PUFAs does not further promote tumors. Ip et al. (10) proposed a similar hypothesis for linoleic acid, and Hopkins and Carroll (11) for PUFAs in general, although Hopkins and Carroll used primarily n-6 PUFAs in their experiment.

(b) We investigate whether the tumor-enhancing effects of saturated fats, monounsaturated fats, and n-6 PUFAs become similar to each other once a certain level of n-6 PUFAs has been reached. This hypothesis has been suggested by Carroll and Hopkins (12) and others (10, 13). Like the first hypothesis, it is motivated by the role of n-6 PUFAs as EFAs.

(c) We investigate whether the effect of n-6 PUFAs is the same for all levels of energy restriction, and in particular whether the tumor promoting effect of dietary fats is dependent on ad libitum feeding. This hypothesis was proposed by Thompson et al. (14) and has been studied specifically by Welsch et al. (15) and Bunk et al. (16).

MATERIALS AND METHODS

Literature Search

For this meta-analysis we have updated a database described in a previous report (1). We have followed procedures similar to the previous methodology in adding more recent articles, but we have also excluded some of the experiments in the original database as a result of using more stringent criteria for inclusion. For example, in the updated database, we require that the source of dietary fat be known because we wish to study the effects of different fatty acids on breast cancer risk. For ease of explanation, we describe our literature search as if it occurred at one time; however, most of the articles published before 1987 come from the original search, whereas those articles published after 1987 result from a second search conducted in 1993.

We used the MEDLINE system to search for articles using the keywords “experimental mammary neoplasms,” “mice or rats,” and “dietary fats.” The search covered the years from 1966 to 1994. Further articles were found from citations in the included articles.

We read abstracts and articles to determine whether the article described an experiment in which mice or rats were randomized to different diets and followed to a point where final tumor incidence in each group was reported. Some articles may describe one or more experiments that have been designed to study other effects besides changes in dietary fat. To use data from these experiments in our analysis, we defined sets of animal groups (from the same experiment) that have been treated the same except for changes in diet, primarily in fat or energy intake. For example, Chan and Dao (17) performed one experiment from which we extracted three sets. Chan and Dao’s experiment comprised nine groups of rats (three different strains each divided into three diet groups) fed a high-fat semipurified diet, a low-fat semipurified diet, or a nonpurified diet. We extracted from the original experiment three sets that compare high-fat to low-fat groups within each strain. We excluded the groups fed nonpurified diets because semipurified and nonpurified diets may have different effects on tumor growth beyond those described by the macronutrient content of the diet (18, 19).

Each set must meet the following criteria:

• The set is (or is part of) a comparative study in mice or rats, in which tumor incidence is reported.

• There are at least two experimental groups fed different amounts or sources of fat.

• Animals are all of the same species and strain.

• Animal groups are followed for the same duration.

• Animals are fed semipurified diets.

• The only interventions (besides the carcinogenic insult) are dietary.

• The sources of fat are reported.

• Animals receive the same carcinogenic insult at the same age.

• The carcinogenic insult is not from transplanted tumors.

• The carcinogen is not administered through the diet.
The experimental diet is fed not later than 5 weeks after carcinogenic insult.

Each animal group receives only one experimental diet (i.e., there are no "crossover" experiments).

The percentage of fiber in the diet is similar between different diet groups within the set. Specifically, the percentage of fiber in the diet by weight for each group is not more than twice the corresponding percentage for any other group in the set.

The data are not reported in another article already in the database.

Throughout this article we use the term "set" as defined above.

In general, we make comparisons only within sets, which are constructed so as to contain animal groups with different fat or energy intakes but with no other, nondietary differences. When groups within a set differ only by type of fat, all other nutrients in the diet are kept the same; however, in other cases, adjustments may be made to the proportions of other nutrients by weight in the diet. For example, in most of the sets with energy-restricted groups, the restricted groups were fed diets in which dietary components were adjusted to maintain adequate nutrient intake. A different adjustment was made in most of the sets with diets of differing proportions of fat but constant energy intake. In these cases, the high-fat diets had a higher proportion of vitamins and minerals by weight than the low-fat diet, so that the proportion of these nutrients by calorie is constant within the set, and all groups receive the same amount of these nutrients. Although sets of these types that do not make these adjustments are included, they are not the majority. Thus, we do not expect that changes in micronutrient intake would explain the effects shown in this paper.

Description of Database

The database comprises data from 146 sets extracted from 97 articles (10, 11, 17, 20–113). The total number of animal groups is 442 and the total number of animals is 12,803.

Of the 146 sets, 124 included groups of rats and 22 included mice. Our primary analysis uses only the rat data; other secondary analyses use only mice, only Sprague-Dawley rats, or all animals combined. In Table 1, we list characteristics of all the sets, of the sets of rats only, and of the sets of Sprague-Dawley rats only.

We see from Table 1 that most of the sets are from experiments in which there is no energy restriction in the diets (i.e., the animals were fed ad libitum or at a level comparable in calories to other animals of that strain fed ad libitum). Furthermore, we note that approximately one-half of the animal groups belong to sets where all groups within the set were fed the same source of fat. The 34 sets in which animal groups were fed different sources of fat are particularly helpful for estimating the effects of different types of fatty acids. The fat in the diet was provided by a variety of fats or oils. In 104 animal groups, different types of fats or oils were mixed to provide the total fat in the diet. In the other 338 animal groups, all of the fat was provided from one source. Of these 338 groups, a substantial majority were fed corn oil (228); in the other 338 animal groups, all of the fat was provided from one source.

In most of the sets, mammary tumors were induced by administration of 7,12-dimethylbenz(a)anthracene (93 sets) or methyl nitrosourea (32 sets). Some of the mouse experiments involved strains that are susceptible to spontaneous mammary tumors (13 sets). Other methods used to induce mammary tumors were: administration of estrone (two sets), radiation (one set), virus from the mother (three sets), administration of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (one set), and administration of N-ethyl-N-nitrosourea (one set). As shown in Table 1, slightly less than one-half of the sets the experimental diets were initiated before the carcinogen administration.

Note that in almost all of these sets the animals were fed the same diets for a short period while the carcinogen was given, and then the experimental diets were resumed. In only six sets, the experimental diets were not resumed after the carcinogen dose.

The duration of the experiment from start of experimental diet or carcinogen until the death or sacrifice of all animals was 10–40 weeks in 122 of the 146 sets and up to 107 weeks in the remainder.

We chose the response variable for each animal group to be tumor incidence. The median tumor incidence from all the animal groups in the full database was 65% with upper and lower quartiles of 83 and 40%. The median size of the animal groups was 30 animals with upper and lower quartiles of 35 and 20.

Statistical Methods

The discussion of the statistical methods is presented in two parts. First, we describe the variables used to measure the dietary intake of fat and energy. Second, we describe the logistic regression method used to relate the risk of mammary tumor to dietary intake.

In a previous meta-analysis (1) we used total calories to measure the effect of energy intake on tumor incidence. To measure the energy effect in this meta-analysis we use percentage of calorie restriction (RESTRICT). The value of RESTRICT is zero in all groups fed ad libitum. Its value is nonzero if the intake is restricted and is equal to the percentage of reduction from the energy consumed on average by an animal of the same strain and species when fed ad libitum.

Most of the articles report the percentage of fat in the diet by weight. We convert this to percentage of calories from fat using the Atwater values (114) of 4, 4, and 9 kcal/g for carbohydrate, protein, and fat, respectively. We assume that all nonfat calories are 4 kcal/g and calculate:

\[
\% \text{ calories from fat} = 100 \times \left( \frac{9 \times \% \text{ fat by weight}}{9 \times \% \text{ fat by weight} + 4 \times (100 - \% \text{ fat by weight})} \right)
\]

Welsch (2, 4) recommends modifying the energy value from fat from 9 to 11.1 kcal/g. The modification does not materially affect the results of our analysis. We therefore use the conventional Atwater values and note that these represent the metabolizable energy, not the recovered energy to which the 11.1 value refers (115, 116).

In most of the experiments, specific sources of fat were added to a semi-purified diet that provided other essential nutrients. Although some of the
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Table 2 Quantiles for diet variables per animal group, full database

<table>
<thead>
<tr>
<th>Variablea</th>
<th>0% (minimum)</th>
<th>25%</th>
<th>50% (median)</th>
<th>75%</th>
<th>100% (maximum)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESTRICT</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>% of calories from fat</td>
<td>0</td>
<td>10.59</td>
<td>29.37</td>
<td>36</td>
<td>66.17</td>
</tr>
<tr>
<td>SAT</td>
<td>0</td>
<td>1.41</td>
<td>4.79</td>
<td>7.04</td>
<td>39.99</td>
</tr>
<tr>
<td>MONO</td>
<td>0</td>
<td>2.68</td>
<td>6.07</td>
<td>10.34</td>
<td>31.55</td>
</tr>
<tr>
<td>N6</td>
<td>0</td>
<td>3.82</td>
<td>6.43</td>
<td>21.85</td>
<td>32.11</td>
</tr>
<tr>
<td>N3</td>
<td>0</td>
<td>0.07</td>
<td>0.14</td>
<td>0.30</td>
<td>12.67</td>
</tr>
</tbody>
</table>

a RESTRICT represents the percentage of calorie restriction. SAT, MONO, N6, and N3 represent the percentage of calories in the diet from the respective type of fat.

articles reported the content of individual fatty acids in each of the fats, many of the articles do not give such information. Thus, we calculated the percentage of fatty acids in the fats from tables of the United States Department of Agriculture (117) for most of the fats used in the experiments. We divided the fatty acid values into four types: saturated fats, monounsaturated fats, n-6 polyunsaturated fats, and n-3 polyunsaturated fats. Then we calculated the total percentage of each type of fatty acid in the diet, creating the four variables.

\[ \text{SAT} = \% \text{ calories from fat} \times \text{ proportion of saturated fat} \]
\[ \text{MONO} = \% \text{ calories from fat} \times \text{ proportion of monounsaturated fat} \]
\[ \text{N6} = \% \text{ calories from fat} \times \text{ proportion of n-6 polyunsaturated fat} \]
\[ \text{N3} = \% \text{ calories from fat} \times \text{ proportion of n-3 polyunsaturated fat} \]

The proportion of fat in each of the categories is calculated either by weight or by calories; the result is the same. Treating each group as an observation, we list the minimum, maximum, median, and 25th and 75th percentiles for the diet variables in the full database in Table 2.

Using these variables, we related the dietary effects to the tumor incidence using a CLRS. We briefly explain this method below. A more detailed presentation of the method is found in Fay et al.3

The primary response for each animal group, the tumor incidence, is the proportion of animals developing one or more tumors during the course of the experiment. Because these primary responses are represented as proportions, an appropriate and common type of model to relate tumor risk to dietary intake is logistic regression (118). For each animal group, the tumor incidence depends not only on dietary intake but also on other factors, which vary from set to set, such as the dose of carcinogen or the time until sacrifice of the animals. Therefore, it is important to account for these set effects in any experiment. Because these primary responses are represented as proportions, the presentation of the method is found in Fay et al.3


Before describing the CLRS method, we describe the simpler unconditional logistic regression model used in Freedman et al. (1) because the diet effects may be interpreted in the same way as for the unconditional logistic model. We prefer the conditional logistic regression over the unconditional one because it does not require the estimation of nuisance parameters. In addition, only with the conditional logistic regression may we use the sandwich estimate of the variance (120) so as to obtain correct estimates of variance even if the dietary effects differ from set to set. In the meta-analysis literature, such differences between sets are known as “random effects,” and the National Research Council report on combining information recommends accounting for random effects whenever possible (121, 122). Thus, the methods we use specifically cater for the meta-analysis of responses given as proportions and perform well if the true model is either a fixed effects one or a random-effects one.

Using the full rat database we form a basic model to describe the effect of different fatty acids and calorie restriction on the log odds of tumor incidence. The formation of the model and the different ways of modeling the (DIET)j effects are discussed in “Results.” After forming this model we check that we obtain similar results from the Sprague-Dawley rats and the other strains of rats. We then apply the model to the mouse data. If the results for mice and rats appear to be compatible, we combine the mouse and rat data to obtain one overall model. In addition to forming this basic model, we test the three specific hypotheses mentioned in the introduction. We discuss these hypotheses more extensively in the next section.

Hypotheses Addressed by the Analysis

Question A: Is There an Intake Level of n-6 Polyunsaturated Fat beyond Which No Further Tumor Promotion Occurs? Certain PUFAs are known as EFAs because animals not fed any of these fatty acids develop deficiency symptoms (44, 123), and these fatty acids cannot be synthesized by animals (124). Often, linoleic acid or linoleate (18:2 n-6) is referred to synonymously with EFA (8, 10) because in many common fat sources (e.g., beef fat, corn oil, and sunflower oil), a substantial majority of the EFA is linoleic acid. Linoleic acid may be metabolized within the body to other longer chain n-6 PUFAs, which can act as EFAs as well (124). All of the n-6 PUFAs measured in our database refer to n-6 EFAs. Some n-3 PUFAs have been classified as EFAs, but their role as an EFA is not as clear (124). In any case, it has been reported that the effects of dietary n-3 and n-6 PUFAs are very different with respect to tumorigenesis (6), so we differentiate between them in our analyses. Stressing the role of linoleic acid as an EFA, Ip et al. (10) hypothesized that beyond a certain saturation point, there is essentially no further promoting effect of linoleic acid on tumors. Hopkins and Carroll (11) proposed a similar hypothesis. In this paper, we investigate this saturation point hypothesis for total n-6 PUFAs.

Ip et al. (10) estimated the saturation point of linoleic acid in the diet to be

\[ \beta_3 \text{(MONO)}_j \]

where (RESTRlCT)j is the percentage of energy restriction and (N6)j, (SAT)j, (MONO)j, and (N3)j are percentages of calories from the respective types of fatty acids for the jth group of the ith set. The parameters are calculated using the logistic regression method. A value of \( \beta_3 \) that is significantly different from zero implies that an increase in energy restriction will decrease the risk of tumor incidence. Interpreting the values for \( \beta_1, \beta_2, \beta_3, \) and \( \beta_4 \) is slightly more complicated because they represent substitution effects (119). For example, a value of \( \beta_1 \) that is significantly greater than zero implies that a substitution of n-6 PUFAs for nonfat calories increases the risk of tumor incidence. We need not pay attention to the (SET) parameters; in statistical terms they are called nuisance parameters.

The CLRS method accounts for the set effects not by estimating them but by conditioning on the total number of animals with tumors within a given set. However, the estimates of the parameters of the (DIET) effects may be interpreted in the same way as for the unconditional logistic model. We prefer the conditional logistic regression over the unconditional one because it does not require the estimation of nuisance parameters. In addition, only with the conditional logistic regression may we use the sandwich estimate of the variance (120) so as to obtain correct estimates of variance even if the dietary effects differ from set to set. In the meta-analysis literature, such differences between sets are known as “random effects,” and the National Research Council report on combining information recommends accounting for random effects whenever possible (121, 122). Thus, the methods we use specifically cater for the meta-analysis of responses given as proportions and perform well if the true model is either a fixed effects one or a random-effects one.

Using the full rat database we form a basic model to describe the effect of different fatty acids and calorie restriction on the log odds of tumor incidence. The formation of the model and the different ways of modeling the (DIET)j effects are discussed in “Results.” After forming this model we check that we obtain similar results from the Sprague-Dawley rats and the other strains of rats. We then apply the model to the mouse data. If the results for mice and rats appear to be compatible, we combine the mouse and rat data to obtain one overall model. In addition to forming this basic model, we test the three specific hypotheses mentioned in the introduction. We discuss these hypotheses more extensively in the next section.
4.4% by weight, corresponding to approximately 8% of calories. They modeled the probability of tumor incidence \( P_{I6} \) to increase linearly with the percentage of linoleic acid up to the saturation point. Animals fed linoleic acid at a level higher than the saturation point had approximately the same tumor incidence as those fed the saturation point level.

We slightly modify the model of Ip et al. (10). As mentioned in “Statistical Methods,” we model the effect of n-6 polyunsaturated fat on the log odds of the probability of tumor incidence. In addition, our model represents a substitution effect of n-6 PUFAs for nonfat calories, whereas Ip et al. (10) created an experimental situation in which they measure a different substitution effect. They mix different levels of corn oil and coconut oil, so that they are essentially measuring the substitution of linoleic acid for saturated fats (because corn oil is mostly linoleic acid, whereas coconut oil is mostly saturated fats). In our model, we control for the variables \( \text{RESTRICT}\), \( N3 \), \( SAT \), and \( MONO \) by including them as terms in the \( \text{DIET} \) part of our model. We create two variables from the \( N6 \) variable so that the n-6 polyunsaturated fat effect is described by two straight lines connected at some “change point” (see Fig. 1). If the slope of the second line is zero, then the change point is a saturation point. If \( C \) is the change point, we define the variables as follows:

\[
\ln N6_C = \begin{cases} 
N6 & \text{if } N6 < C \\
C & \text{if } N6 \geq C
\end{cases}
\]

and

\[
\text{UN6}_C = \begin{cases} 
0 & \text{if } N6 < C \\
N6 - C & \text{if } N6 \geq C
\end{cases}
\]

The change point model is then described as follows:

\[
(\text{DIET})_{ij} = \beta_0(\text{RESTRICT})_{ij} + \beta_1(N3)_{ij} + \beta_2(SAT)_{ij} + \beta_3(MONO)_{ij} + \beta_4(\text{LN6}_C)_{ij} + \beta_5(\text{UN6}_C)_{ij}
\]

where \( \beta_0, \beta_1, \beta_2, \beta_3, \beta_4, \beta_5 \) are the coefficients for the adjusting variables, \( \beta_0 \) represents the slope of the N6 effect up to the change point, and \( \beta_1, \beta_2, \beta_3 \) represent the slope of the N6 effect beyond the change point. If there were no further increase in the probability of tumor incidence after the change point, \( \beta_1 \) would be zero, and \( C \) would represent a saturation point. We estimate the change point by finding the value \( C \) that best fits the data and then formally test whether \( \beta_5 = 0 \) using the CLRS method. If \( \beta_5 \) is significantly different from zero, then we conclude that there is no plateau after the change point value.

**Question B: Do Saturated, Monounsaturated, and n-6 Polyunsaturated Fats Have a Similar Effect beyond a Certain Level of n-6 Polyunsaturated Fat?** In question A, we hypothesized that n-6 PUFAs act differently with respect to mammary tumor risk up to and beyond some change point for n-6 PUFAs. Question B is a modification of question A and is motivated by the idea that in addition to the n-6 PUFAs, the saturated and monounsaturated fats also act differently up to and beyond the change point value of n-6 PUFAs. The associated hypothesis states that after some change point value of n-6 PUFAs intake is exceeded, saturated and monounsaturated fats have the same tumor-promoting effect as n-6 PUFAs. This hypothesis has been mentioned previously in the literature by Carroll and Hopkins (12) and others (13, 10).

We again define the change point as \( C \), which is estimated from the previous models. To test this hypothesis, we first choose the animal groups that have N6 values greater than \( C \) and have at least one other animal group within its set with N6 values greater than \( C \) as well. Then, with this smaller data set, we model the \( \text{DIET}_{ij} \) effect as follows:

\[
(\text{DIET})_{ij} = \beta_0(\text{RESTRICT})_{ij} + \beta_1(N3)_{ij} + \beta_2(SAT)_{ij} + \beta_3(MONO)_{ij} + \beta_4(N6)_{ij}
\]

We then test whether \( \beta_2 = \beta_3 = \beta_4 = 0 \). If \( \beta_2 \) is significantly different from \( \beta_3, \beta_4 \), then we infer that the saturated fat effect is different from the n-6 PUFAs effect after the change point. Similarly, if \( \beta_3 \) is significantly different from \( \beta_4 \), then we say that the monounsaturated fat effect is different from the n-6 PUFAs effect after the change point.

**Question C: Is There an Interaction between n-6 Polyunsaturated Fats and Total Energy Intake?** Thompson et al. (14) have suggested that the promoting effect of fat on mammary tumors is dependent on ad libitum feeding. Further studies have investigated this issue (15, 16). Two of these studies (14, 15) used corn oil, which has a high n-6 PUFAs content. Using our database, we test a related hypothesis by testing for an interaction between the intake of n-6 PUFAs and the percentage of restriction of the diet. Thus, our \( \text{DIET}_{ij} \) effect is modeled as follows:

\[
(\text{DIET})_{ij} = \beta_0(\text{RESTRICT})_{ij} + \beta_1(N3)_{ij} + \beta_2(SAT)_{ij} + \beta_3(MONO)_{ij} + \beta_4(N6)_{ij} + \beta_5(\text{RESTRICT})_{ij}(N6)_{ij}
\]

If the promoting effect of n-6 PUFAs is stronger for ad libitum-fed animals and restriction of energy reduces the effect of n-6 PUFAs, then we would expect that \( \beta_5 \) would be negative. Alternatively, if the promoting effect of n-6 PUFAs is the same for all levels of diet restriction, then \( \beta_5 = 0 \). We test whether \( \beta_5 = 0 \) using the CLRS model.

**RESULTS**

**Modeling the Diet Effects.** We model the \( \text{DIET}_{ij} \) effects using the CLRS method as described in “Statistical Methods” section. We first form a basic model on the data of the rats only, with a main effects term for each of the dietary intake variables.

\[
(\text{DIET})_{ij} = \beta_0(\text{RESTRICT})_{ij} + \beta_1(N3)_{ij} + \beta_2(SAT)_{ij} + \beta_3(MONO)_{ij} + \beta_4(N6)_{ij}
\]

The parameter estimates and confidence intervals are listed in Table 3 as model 1. We first note the direction of the effects; increasing percentage of energy restriction and percentage of n-3 PUFAs decreases the risk of mammary tumors, whereas increasing percentage of saturated, monounsaturated, and n-6 fats increases the risk of mammary tumor.

The effect of energy restriction is not quite statistically significant at the 0.05 level. This is primarily because the data set is not optimally designed to detect those effects; we have only 13 sets with energy restriction, and the CLRS method is conservative when there is a small number of sets. Failure to achieve statistical significance does not imply that the effect does not exist, only that this analysis does not
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Table 3: Models 1 and 2 on all rats

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Parameter estimate</th>
<th>95% confidence limits</th>
<th>Parameter estimate</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESTRICT</td>
<td>−0.069</td>
<td>(−0.139, 0.000)</td>
<td>−0.068</td>
<td>(−0.136, −0.001)</td>
</tr>
<tr>
<td>N3</td>
<td>−0.019</td>
<td>(−0.083, 0.045)</td>
<td>−0.013</td>
<td>(−0.076, 0.050)</td>
</tr>
<tr>
<td>SAT</td>
<td>0.0009</td>
<td>(−0.003, 0.020)</td>
<td>0.014</td>
<td>(0.000, 0.026)</td>
</tr>
<tr>
<td>MONO</td>
<td>0.024</td>
<td>(0.006, 0.041)</td>
<td>0.017</td>
<td>(−0.001, 0.035)</td>
</tr>
<tr>
<td>N6</td>
<td>0.055</td>
<td>(0.045, 0.065)</td>
<td>0.199</td>
<td>(0.124, 0.275)</td>
</tr>
<tr>
<td>LN6₄</td>
<td>0.049</td>
<td>(0.039, 0.059)</td>
<td>0.0001</td>
<td></td>
</tr>
</tbody>
</table>

* All models relate log odds of mammary tumor incidence to dietary factors. Positive parameter estimates denote higher predicted incidence for larger values of the covariate.

Conclusively establish its existence (125). We similarly have nonsignificant or marginally significant results for the N3, SAT, and MONO effects. However, the N6 effect is strongly significant (P < 0.0001).

To explore the N6 effect further, we model the N6 effect with the two parameters, LN6₄ and UN6₄, described in “Hypothesis.” In this model, N6 has one effect before C and a different effect after C (see Fig. 1). We try different integer values of C ranging from 2 to 21% n-6 PUFAs. Consider the two nonsignificant effects from the combined analysis. Part of the reason we cannot detect the effect for n-3 PUFAs is because few of the experiments used substantial amounts of n-3 PUFAs (see Table 2).

We consider the effects from the four significant variables, RESTRICT, SAT, LN6₄, and UN6₄. To aid in the interpretation of the parameter estimates of the combined data model, we consider the parameter estimates for the N6 effect with the 95% confidence intervals in the last two columns of Table 5. The effect from N6, both before and after C = 4%, is larger than the effect of SAT, MONO, and N3. Further, the effects for RESTRICT, SAT, and UN6₄ are significant at the 0.05 level.

Consider the two nonsignificant effects from the combined analysis. The MONO effect is in different directions for the rats alone and the mice alone, so the nonsignificant result for the combined data is expected. Although the estimates for the n-3 effects are in the same direction for both rats and mice, the associated P values are far from significant; (P = 0.62) for rats and (P = 0.62) for mice. Thus, the nonsignificant result for N6 from the combined data (P = 0.51) is not surprising. None of the three analyses definitively support the hypothesis that n-3 PUFAs are protective against mammary tumors, but they also do not disprove this hypothesis. Part of the reason we cannot detect the effect for n-3 PUFAs is because few of the experiments used substantial amounts of n-3 PUFAs (see Table 2).

To give a visual impression of the n-6 PUFAs results, we divided the N6 variable into 10 ordered categories (see Ref. 126) and plotted the N6 parameter estimate in each category with 95% confidence intervals. The direction for both rats and mice, the associated P values are far from significant; (P = 0.62) for rats and (P = 0.62) for mice. Thus, the nonsignificant result for N6 from the combined data (P = 0.51) is not surprising. None of the three analyses definitively support the hypothesis that n-3 PUFAs are protective against mammary tumors, but they also do not disprove this hypothesis. Part of the reason we cannot detect the effect for n-3 PUFAs is because few of the experiments used substantial amounts of n-3 PUFAs (see Table 2).

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Table 4: Model 2 on different strains of rats

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Parameter estimate</th>
<th>95% confidence limits</th>
<th>Parameter estimate</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESTRICT</td>
<td>−0.055</td>
<td>(−0.135, 0.024)</td>
<td>−0.127</td>
<td>(−0.241, −0.012)</td>
</tr>
<tr>
<td>N3</td>
<td>−0.0003</td>
<td>(−0.078, 0.077)</td>
<td>−0.040</td>
<td>(−1.318, 1.237)</td>
</tr>
<tr>
<td>SAT</td>
<td>0.018</td>
<td>(0.001, 0.036)</td>
<td>0.002</td>
<td>(−0.025, 0.029)</td>
</tr>
<tr>
<td>MONO</td>
<td>0.018</td>
<td>(−0.010, 0.046)</td>
<td>0.015</td>
<td>(−0.014, 0.043)</td>
</tr>
<tr>
<td>LN6₄</td>
<td>0.205</td>
<td>(0.119, 0.290)</td>
<td>0.146</td>
<td>(−0.677, 0.970)</td>
</tr>
<tr>
<td>UN6₄</td>
<td>0.049</td>
<td>(0.035, 0.065)</td>
<td>0.054</td>
<td>(0.040, 0.069)</td>
</tr>
</tbody>
</table>

* All models relate log odds of mammary tumor incidence to dietary factors. Positive parameter estimates denote higher predicted incidence for larger values of the covariate.

For the mouse data, we again fit model 2. In this case, we fix C at 4% of n-6 PUFAs because the change point is hard to estimate, and there are many fewer data for mice.

The parameter estimates for model 2 applied to the mouse data are listed in the second row of Table 5 (we provide the estimates for the rat data in the first column for comparison). Because there are substantially fewer data for the mice, none of the effects are statistically significant except the calorie restriction effect. As expected, calorie restriction decreases tumor risk. Although none of the fat effects are significant, all of the effects except that for monounsaturated fats are in the same direction as for the rat data. Recall that the monounsaturated fat effect is not significant in the rat data. Also note that every parameter estimate in the rat data falls within the confidence intervals for the mouse data. Thus, it is plausible that one model holds for both species.

We combine the rat and mouse data and run model 2 again. We list the parameter estimates with confidence intervals in the last two columns of Table 5. The effect from N6, both before and after C = 4%, is larger than the effect of SAT, MONO, and N3. Further, the effects for RESTRICT, SAT, and UN6₄ are significant at the 0.05 level.

Consider the two nonsignificant effects from the combined analysis. The MONO effect is in different directions for the rats alone and the mice alone, so the nonsignificant result for the combined data is expected. Although the estimates for the n-3 effects are in the same direction for both rats and mice, the associated P values are far from significant; (P = 0.62) for rats and (P = 0.62) for mice. Thus, the nonsignificant result for N6 from the combined data (P = 0.51) is not surprising. None of the three analyses definitively support the hypothesis that n-3 PUFAs are protective against mammary tumors, but they also do not disprove this hypothesis. Part of the reason we cannot detect the effect for n-3 PUFAs is because few of the experiments used substantial amounts of n-3 PUFAs (see Table 2).
Table 5 Parameter estimates for Model 2

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Rat data</th>
<th>95% confidence limits</th>
<th>Mouse data</th>
<th>95% confidence limits</th>
<th>Rat and mouse combined</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>RESTRICT</td>
<td>-0.068$^e$ (-0.136, -0.001)</td>
<td>-0.104$^e$ (-0.160, -0.048)</td>
<td>-0.082$^e$ (-0.127, -0.036)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N3</td>
<td>-0.013 (-0.076, 0.050)</td>
<td>-0.075 (-0.462, 0.313)</td>
<td>-0.036 (-0.175, 0.103)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAT</td>
<td>0.014$^e$ (0.001, 0.026)</td>
<td>0.027 (-0.035, 0.089)</td>
<td>0.017$^e$ (0.005, 0.028)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MONO</td>
<td>0.017 (-0.001, 0.035)</td>
<td>-0.023 (-0.067, 0.022)</td>
<td>0.011 (-0.007, 0.028)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LN64</td>
<td>0.199$^e$ (0.124, 0.275)</td>
<td>0.506 (-0.011, 1.023)</td>
<td>0.264$^d$ (0.116, 0.412)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UN64</td>
<td>0.049$^e$ (0.039, 0.059)</td>
<td>0.044$^e$ (0.001, 0.086)</td>
<td>0.049$^e$ (0.039, 0.058)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ All models relate log odds of mammary tumor incidence to dietary factors. Positive parameter estimates denote higher predicted incidence for larger values of the covariate. Model 2 has:

$$(\text{DIET})_i = \beta_0(\text{RESTRICT})_i + \beta_1(N3)_i + \beta_2(SAT)_i + \beta_3(MONO)_i + \beta_4(LN64)_i + \beta_5(UN64)_i$$

$^b$ RESTRICT is the percentage of calorie restriction. SAT, MONO, and N3 are the percentage of calories in the diet from the respective type of fat. LN64 represents n-6 PUFAs less than 4% of calories, and UN64 represents n-6 PUFAs greater than 4% of calories.

$^c$ Parameter estimate significantly different from 0; $P < 0.05$.

$^d$ Parameter estimate significantly different from 0; $P < 0.01$.

$^e$ Parameter estimate significantly different from 0; $P < 0.0001$.

We now address the three hypotheses mentioned previously, using data on both the rats alone and on the combined rats and mice.

**Question A: Is There an Intake Level of n-6 Polyunsaturated Fat beyond Which No Further Tumor Promotion Occurs?** In “Hypothesis,” we discussed testing this hypothesis using the model that we have called model 2 above. We have seen in Table 5 that the parameter for UN64 is significantly different from zero for both the rat data and the combined data. The $P$ for this test associated with both the rat and the combined data is $P < 0.0001$. Thus, although there appears to be a stronger effect of n-6 PUFAs at low levels (probably related to its role as an essential fatty acid), there continues to be a significant tumor-promoting effect of N6 even at values larger than 4% of the diet. Note also that this latter effect appears larger than the effects of the saturated fats and the monounsaturated fats. We achieve similar results for change point values from 5 to 8.

**Question B: Do Saturated, Monounsaturated, and n-6 Polyunsaturated Fats Have a Similar Effect beyond a Certain Change Point of n-6 Polyunsaturated Fat?** First, we analyzed data from the rats alone. Using a change point value of 4% n-6 PUFA, we created a smaller database of only those sets that had at least two groups of rats fed diets containing over 4% n-6 PUFA. This reduced database comprises data from 88 sets and 240 groups of rats. We formed a model using main effects terms for RESTRICT, N3, SAT, MONO, and N6 and obtained results listed in the first column of Table 6. As in model 1 of Table 3, the parameter estimate corresponding to N6 was much larger than the estimates for SAT and MONO. As explained in “Hypothesis,” we tested whether the SAT effect or the MONO effect was significantly different from the N6 effect after the change point value of $C = 4%$ of n-6 PUFAs was met. We found that the effect for saturated fats was significantly lower than the effect for n-6 PUFAs ($P = 0.001$), whereas the effect for monounsaturated fats was lower but not statistically significantly different ($P = 0.375$).

Next, we repeated the analysis after adding 14 sets of mouse data comprising 39 animal groups. The results are listed in the second column of Table 6. Not much is changed by the addition of the mouse data: we still found that the saturated fat effect is significantly lower than the effect for n-6 PUFAs ($P = 0.002$), and there is no significant difference between the monounsaturated effect and the n-6 PUFA effect ($P = 0.775$).

**Question C: Is There an Interaction between n-6 Polyunsaturated Fats and Total Energy Intake?** When we tested the interaction model on the rat data, we found a nonsignificant negative inter-

![Fig. 2. Parameter estimates with 95% confidence limits (bars) for each decile of percentage of calories from n-6 PUFA; rats and mice combined.](cancerres.aacrjournals.org)
action term \((P = 0.117)\). Thus, although there is an indication that the n-6 PUFAs effect is strongest for diets without energy restriction, this result is not statistically significant. Adding the mouse data, we still found a negative interaction term, but the significance is diminished \((P = 0.554)\). For this hypothesis, we performed a test on the mouse data alone because in this species Freedman et al. \((1)\) found a highly significant interaction between the fat effect and restricted diets. However, when we performed the test we found a nonsignificant positive interaction \((P = 0.698)\). The direction of the effect agrees with Freedman et al. \((1)\), but the Ps are very different. The difference is due to the use of the CLRS method, which allows for between-experiment variation in the dietary effects and is more conservative than the fixed effects method used by Freedman et al. \((1)\).

As stated in "Statistical Methods," the CLRS method is the more appropriate method.

**DISCUSSION**

Over the past 10 years there have been many extensive "qualitative" reviews published on the effects of different types of dietary fats on mammary tumorigenesis in experimental animals \((2-6, 127-129)\) in which reviewers select the experiments they consider most important and use the results from these to draw conclusions. The advantage of the "quantitative" review (meta-analysis) we present here is that we include all reported experiments that meet certain objective criteria. In addition, we are able to model the effects of several types of fatty acids simultaneously and to obtain specific quantitative estimates of their effects. As discussed in Freedman et al. \((1)\), by including experiments that investigate the same effects under different experimental designs, we may have more confidence in the generality of the results. Thus, we are able to answer in a more definitive way certain hypotheses about the way different fatty acids affect mammary tumor incidence.

Experiments by both Ip et al. \((10)\) and Hopkins and Carroll \((11)\) suggest that there is some saturation point of linoleic acid beyond which the addition of more linoleic acid acts similarly to other fatty acids. We have combined these data with data from 95 other reports to explore the saturation point hypothesis further.

Because recent research has suggested that linoleic acid acts on tumor enhancement through elongation to arachidonic acid \((20:4, n-6; \text{Ref. } 5)\), we use one term for linoleic acid, arachidonic acid, and all other n-6 PUFAs. We found that n-6 PUFAs enhance tumors compared to nonfat calories. We found that at levels of dietary n-6 PUFAs less than 4% of calories there is a stronger tumor-enhancing effect than at levels over 4%. We modeled the effect as linear effects in the log odds of tumor incidence. Although the change point of the linear effect of n-6 PUFAs was best estimated as 4% calories from the rat data, other values may be plausible. The best estimate of the change point for the mice was 7% of calories, and for mice and rats combined, it was 5% of calories. In fact, plausible change point values range from 4 to 8% calories from n-6 PUFAs. Other models are plausible as well. For example, one alternative is that n-6 PUFAs tumor-enhancing effects change smoothly with n-6 PUFAs levels, with a rapid increase in log odds of tumor at low n-6 levels and a slower increase at high n-6 levels. However, any plausible model would agree on two aspects of the n-6 PUFAs effect. First, n-6 PUFAs increase the log odds of tumor more rapidly at lower levels than at higher levels. Second, there is still a tumor-enhancing effect for n-6 PUFAs in comparison to nonfat calories even at the higher levels.

It has been generally agreed that the tumor-enhancing effect of saturated fats is less than that of n-6 PUFAs \((4)\). But Ip et al. \((10)\) and others have hypothesized that this difference may be due only to the lack of essential fatty acids in saturated fat sources. We have shown that saturated fat calories enhance tumors more than nonfat calories but less than n-6 PUFAs. This is true at any level of n-6 PUFAs, whether less than or greater than 4% of calories. This result does not support the hypothesis of Ip et al. \((10)\). Furthermore, there is no indication from our analyses that the effects of saturated fat are greater in the presence of more than 4% n-6 PUFAs than in the absence of this level of n-6 PUFAs. Results relating to monounsaturated fats are inconclusive in regard to their effects in the presence of more than 4% n-6 PUFAs.

Thompson et al. \((14)\) and Welsch et al. \((15)\) have performed experiments suggesting that rats fed *ad libitum* show a stronger tumor promoting effect from corn oil (a fat primarily composed of n-6 PUFAs) than rats on energy restricted diets. Our data do suggest overall that the tumor-enhancing effect of n-6 PUFAs on rats is stronger when there is no energy restriction, but the results are not significant. The direction of the result agrees with the results of Thompson et al. \((14)\) and Welsch et al. \((15)\). Neither of those two reports met our inclusion criteria; in the Thompson et al. \((14)\) experiment, each animal group received more than one experimental diet, and in the Welsch et al. \((15)\) experiment, the average number of tumors but not tumor incidence was reported. Thus, the available evidence for interaction may be stronger than our analysis suggests. When we performed the same analysis on the mice, the data suggested the opposite effect: the n-6 PUFAs effect was stronger with energy restriction, although this effect was also not significant. Overall evidence for this interaction, therefore, remains inconclusive.

In our models, the parameters for each of the four types of fats represent a substitution of that type of fat for a nonfat calorie. In
general, care should be taken in interpreting effects derived from dietary experiments. For example, the important experiment by Ip et al. (10) is often cited focusing primarily on the effect of linoleic acid on tumor enhancement (2, 19, 127). But Ip et al. (10) created increasing levels of dietary linoleic acid by mixing corn oil (high in linoleic acid) and coconut oil (high in saturated fats) in different amounts. Thus, the linoleic acid effect shown by Ip et al. (10) is essentially a substitution effect of linoleic acid for saturated fat (or vice versa). This result is expressed in our models through the fact that the coefficient for n-6 PUFA is substantially larger than the coefficient for saturated fat.

The substitution interpretation is also important for understanding previous experiments on n-3 PUFA effects. For example, experiment 1 of Jurkowski and Cave (76) increased the percentage of menhaden oil (high in n-3 PUFA) in the diet, substituting menhaden oil for nonfat calories. However, a different type of substitution was used in the experiment of Abou-El-Ela et al. (22), in which the animals were fed the same total amount of fat, but in one group the fat was 100% corn oil, whereas in another group the fat was 75% menhaden oil and 25% corn oil. Here, the menhaden oil was substituted for corn oil. Care is needed in comparing the results of these experiments, also, because menhaden oil contains significant amounts of monounsaturated and saturated fats.

The results from our analyses show that substituting n-3 PUFA for nonfat calories may have a protective effect, but the data are not strong enough to establish that effect. A recent review by Noguchi et al. (5) states that n-3 PUFA suppress mammary tumors. To support this hypothesis, they cite an experiment by Karmali et al. (130) involving transplanted tumors; this experiment is not included in our database because this animal model precludes the gathering of incidence data. Thus, the total evidence supporting this hypothesis is stronger than in our analysis but is still sparse compared to the evidence on the effects of n-6 PUFA.

Although our analyses focus on tumor incidence, dietary fats may affect other aspects of tumor growth, such as time to first tumor, tumor size, or number of tumors per animal. Thus, there may be important effects of dietary fats on some of these other measures that cannot be addressed by our analyses.

The role of dietary fat in human breast cancer is still unresolved. International ecological studies that compare food disappearance data with age-standardized breast cancer incidence agree qualitatively with the main results presented here (131, 132). These data show that the types of fat with the largest parameter estimates through regression on the breast cancer incidence are PUFA (primarily n-6 PUFA; see Ref. 132). The next largest is saturated fats, and monounsaturated fats have little effect. On the other hand, Howe et al. (133), combining 12 case-control studies, show that the largest relative risks for breast cancer come from saturated and monounsaturated fats, not polyunsaturated fats. Furthermore, Hunter et al. (134) pooled seven prospective studies and were not able to detect any significant relationship between breast cancer and any type of fat, neither saturated, monounsaturated, nor polyunsaturated fat. One factor in the inconsistency between these studies on humans is the great difficulty of measuring an individual’s dietary intake over a long time with any accuracy (135, 136). Thus, there is a need for large clinical trials to address this issue in humans. Until more is known about the human situation, animal experiments will continue to be of interest, and a broad quantitative approach to their interpretation is to be encouraged.

ACKNOWLEDGMENTS

We thank Tom Riley, who helped with data preparation, and Drs. William T. Cave and Kenneth K. Carroll for comments on the paper.
EFFECT OF FATS ON THE DEVELOPMENT OF MAMMARY TUMORS


Effect of Different Types and Amounts of Fat on the Development of Mammary Tumors in Rodents: A Review


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