LAURENCE S. FREEDMAN*
Biometry Branch
CAROLYN K. CLIFFORD
Diet and Cancer Branch
Cancer Prevention Research Program
Division of Cancer Prevention
and Control
National Cancer Institute
Bethesda, Md

References


Response

I appreciate the comments of Freedman and Clifford in which they question our interpretation of a portion of our data recently published (1). In our study, we concluded that high-fat diets can enhance mammary gland carcinogenesis only in animals (female Sprague-Dawley rats) on an ad libitum feeding protocol and that a mild restriction in the amount of food consumed (12% less than ad libitum) abolished the mammary carcinogenesis differential between a high- and a low-fat diet. After Freedman and Clifford performed statistical tests on the results published in our paper, they believed that a more reasonable conclusion would be “that the fat effect may be greater under ad libitum feeding than under restricted feeding.”

Although Freedman and Clifford have raised a number of valid points, we are not compelled by their comments to markedly change our original interpretation. We will concede, however, that the word “abolished” may be somewhat strong (their major concern). In essence, we continue to support the following statement. When carcinogen-treated female Sprague-Dawley rats are fed low- and high-fat (corn oil) diets, at a level 12% less than ad libitum, no significant (P > .05) difference in the two levels of fat on mammary carcinoma number or mammary carcinoma weight is observed. This was demonstrated in two separate experiments, each experiment using more than ample numbers of rats per group (38 to 42). The main point of our paper was to demonstrate that when one feeds low- and high-fat (corn oil) diets (12% restricted) to carcinogen-treated female rats, one does not achieve differences in mammary carcinoma number or weight that reach the 5% level of statistical probability. The comments of Freedman and Clifford have not caused us to change our position.

In our study, two standard end points of mammary carcinogenesis were used, ie, mammary carcinoma number (mammary carcinomas per rat) and mammary carcinoma weight (mammary carcinoma weight per rat). In the rats fed the 12% restricted diets, mammary carcinoma numbers (mean ± SE) for animals fed low- and high-fat diets were 3.0 ± 0.3 and 4.1 ± 0.5, respectively, in experiment 1 and 3.1 ± 0.4 and 3.7 ± 0.3, respectively, in experiment 2. In both experiments, mean differences in the number of mammary carcinomas per rat in the low- and high-fat diet groups did not reach the 5% level of statistical probability (Student's t test). The mean carcinoma weights per rat in animals fed the low- and high-fat diets were (g ± SE) 3.0 ± 1.1 and 4.6 ± 1.3, respectively, in experiment 1 and 4.3 ± 1.2 and 4.0 ± 1.1, respectively, in experiment 2. Again, in both experiments, the mean difference in mammary carcinoma weight per rat in the low- and high-fat diet groups did not reach the 5% level of statistical probability (Student's t test). When Freedman and Clifford combined the data of experiment 1 and experiment 2, they concluded that the numerical increase in number of mammary carcinomas per rat in the animals fed the high-fat diet, compared with that in the animals fed the low-fat diet, was significant (t = 2.21; P = .03). The combined evidence for significance of number of mammary carcinomas per rat given by Freedman and Clifford (P = .03) does not agree with the widely accepted Fisher criterion (2) that we utilized in our study, which yields a P value of .08. Fisher's method for combining independent tests has been shown to be statistically optimal and admissible (3). Furthermore, Marden (4) has shown that the inverse normal procedure is inadmissible. It is clear, therefore, that when one analyzes our data, either as single experiments or as combined experiments, a difference between low- and high-fat diets on mammary gland tumorogenesis cannot be demonstrated at the 5% level of statistical probability when the animals are fed a mildly restricted diet (12% less than ad libitum). Of the two parameters of mammary tumorigenesis used in our study, neither reached the 5% level of statistical probability: one of these parameters (mammary carcinoma weight per rat) was not even close to this statistical criterion when the experiments were combined. Thus, to precisely communicate our interpretation of our data, I would conclude the following: In carcinogen-treated female Sprague-Dawley rats, feeding 12% restricted (12% less than ad libitum) low- and high-fat (corn oil) diets, the high-fat diet does not increase mammary carcinoma number (multiplicity) or mammary carcinoma weight at the 5% level of statistical probability (P > .05). Although a fat effect may be present in the animals of this study, it cannot be detected at the 5% level of statistical probability.

Freedman and Clifford raise one additional concern, albeit minor, that requires a response. They are curious as to “why the groups of rats in the second experiment began the ‘treatment’ weighing, on average, less than any of the groups in experiment 1 (Table 3), ate fewer calories during treatment (Table 2), but weighed more by the end of treatment (Table 3).” The animals that we used in our study were outbred Sprague-Dawley rats. Variations from batch to batch can be considerable, a phenomenon well recognized by those who use this very popular strain of experimental animal. For example, the animals in experiment 1 (batch 1) and experiment 2 (batch 2) were 60 days old at the time of treatment onset; mean body weights at 60 days of age in the animals of experiment 1 and experiment 2 were 173/174 g and 152 g, respectively. There was a year interval between the purchase of animals of batch
1 and batch 2. Such a discrepancy, however, does not whatsoever compromise the results of our study.

Freedman and Clifford in a recent communication (5) examined the issue as to whether or not the enhancing effect of fat in experimental mammary gland tumorigenesis is dependent on the level of caloric intake. They concluded after reviewing approximately 100 published animal experiments that "there is a specific enhancing effect of dietary fat, as well as a general enhancing effect of calories" in this tumorigenic process. I should note that we arrived at a similar conclusion in a review published a few years earlier (6).

In the study under discussion (1), we were able to demonstrate very clearly that the enhancing effect of dietary fat depends on the level of caloric intake, at least in one experimental animal model, i.e., the carcinogen-treated female Sprague-Dawley rat. To date, debate continues as to whether or not the stimulatory effect of a high-fat diet in experimental mammary gland tumorigenesis is due to a specific effect of fat or to the calories derived from this nutrient. Definitive experiments to resolve this timely and important issue have yet to be performed (reported).

CLIFFORD W. WELSCH*
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, Mich

References

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*Correspondence to: Clifford W. Welsch, PhD, Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.
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