[CANCER RESEARCH 57, 4162–4163, October 1, 1997]

Advances in Brief

Multigenerational Effects of Dietary Fat Carcinogenesis in Mice[1]

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Abstract

The possibility of multigenerational transmission of a carcinogenic effect from exposure to a maternal diet high in fat was tested in mice. Diets with 2.6 or 29% fat (by weight) were fed to strain CD-1 mice during pregnancy. The female offspring were raised on a control diet (10% fat), mated, and continued on the control diet through pregnancy. Their female offspring were raised to terminal illness and autopsied. The total number of reproductive system tumors, pituitary tumors, and metastases was increased in the offspring with ancestral exposure to high dietary fat but to a lesser extent than had been reported previously for direct prenatal exposure to high maternal dietary fat. Because previous work has given evidence against germ cell transmission, a hypothesis based on a maternal effect was offered to explain the multigenerational carcinogenesis. These results have implications for epidemiological studies.

Introduction

The frequency of breast, corpus uteri, and ovarian cancer varies widely between countries, and these differences parallel differences in levels of dietary fat (1, 2). However, adult consumption of fat within a country does not parallel the frequency of breast cancer (3, 4). The alternative is that the sensitive period is earlier in life. Experiments on mice and rats have shown an increase in tumors among the offspring of dams on a high fat diet during pregnancy compared to offspring of dams on a low fat diet (5, 6). This result is consistent with evidence of a higher rate of breast cancer in the offspring of Japanese migrants to California compared to their parents (7). Because the rate of breast cancer can be five times as great in a country with a high fat diet compared to a country with a low fat diet, the potential for cancer reduction in the next generation by reducing fat intake during pregnancy is very great. An appropriate question concerning this potential reduction is whether there would be any multigenerational carry-over of the high fat carcinogenesis, because carry-over effects of another nutritionally related condition have already been reported in a human population (8). This issue would be important to the interpretation of epidemiological studies if populations converting from high fat to low fat diets could be found. Also, the multigenerational behavior of high fat carcinogenesis might contribute information relevant to understanding the carcinogenic mechanism, as in the case of DES[3] multigenerational carcinogenesis (9). Therefore, we have looked for evidence of a carcinogenic effect in the offspring of dams exposed prenatally to a maternal high fat diet but maintained on a low fat diet during their own pregnancy.

Materials and Methods

Strain CD-1 mice (Charles River, Wilmington, MA) were bred, and their offspring were raised on a commercial pelleted diet with 10% fat, by weight. At 4 weeks of age, the female offspring were placed on a powdered diet with either 2.6% or 29% fat from corn oil and other ingredients as described previously (10). These females were mated to control males (*i.e.*, males exposed only to the commercial pelleted diet) and returned to the pelleted diet (10% fat) when their litters were delivered. Females from these litters were raised on the pelleted diet and mated to control males. Their female offspring were raised on the pelleted diet and euthanized with carbon dioxide when terminal illness was recognized by criteria such as palpable tumors or delayed righting reflex.

Mammary gland areas, thoracic and abdominal cavities, and the hypophysial fossa were inspected, and tumors were recorded. Tumors of the mammary gland, reproductive tract, and pituitary gland were removed. In addition, the uterine horns, cervix, and vagina were cut longitudinally for further evidence of tumors. When tumors in any of these organs were seen, then lungs and liver tumors were processed for histological evidence of metastases. All ovaries were processed, because tumors of this organ are often hard to recognize grossly. Tissues were fixed in 10% buffered formalin and processed for histological observation as described previously (9). Uterine adenocarcinomas were distinguished from adenomyosis using histological criteria supplemented by area measurements (11).

Statistical analysis was by the two-tailed t test for age comparison and by the χ^2 method for differences in tumor frequency. The latter was based on sites at risk, specifically bilateral for mammary, ovarian, and uterine horn tumors, and single for pituitary tumors. Metastases are listed as single occurrences per lung rather than counting individual metastatic sites. Means are presented as ± SE.

Results and Discussion

The average age at terminal illness was 22.98 ± 0.62 months, with a range of 13 to 32 months for mice with ancestral exposure to low fat. The average age for mice with ancestral exposure to high fat was 20.8 ± 0.75 months with a range of 4 to 33 months. These age distributions differed significantly ($P < 0.05$). The frequency of tumors in these two groups is shown in Table 1. The total for ovarian, uterine, and mammary tumors is 18 for low fat and 30 for high fat ancestral exposure, which is not significant ($P > 0.1$). This is in contrast to past experiments with direct prenatal exposure to dietary fat in which exposure to high fat produced significantly more tumors of these organs (5, 11). If pituitary tumors and metastases are included in the tally, then there are significantly more tumors in the mice with high fat ancestry than in those with ancestral exposure to low fat (Table 1). Inclusion of these two types of tumors is justified by past evidence on the tendency for pituitary tumors to be more common after high fat exposure and for metastatic tumors to be uncommon except after prenatal exposure to high fat (5, 11).

A reasonable conclusion is that tumor frequency is substantially reduced one generation after prenatal exposure to a maternal high fat diet, but that there is some carry-over of carcinogenic effect. Thus, epidemiological studies of populations switching from high fat in the maternal diet to low fat would not be expected to show as great a difference in frequency of reproductive system tumors as is seen between countries with stable use of these types of diets. The evidence that there is a low level of carcinogenic carry-over to the generation

Received 6/9/97; accepted 8/18/97.

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[1] This study was funded by NIH Grant RO1 CA39456. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

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[3] The abbreviation used is: DES, diethylstilbestrol.

Table 1 *Frequency of diet-related tumors in third generation mice*

Ancestral exposure	No. of tumors					Total tumors	Total mice
	Ovarian	Uterine	Mammary	Metastatic[a]	Pituitary		
Low fat	3	1	14	2	1	21	62
High fat	7	7	16	7	3	40[b]	66

[a] Number of mice with metastatic mammary tumors in lungs.
[b] Significantly increased compared to low fat ancestry ($P < 0.05$).

without direct high fat exposure provides some evidence for the mechanism of carcinogenesis from prenatal exposure to high fat. It can be contrasted to the multigenerational effect of DES, where a significant effect on reproductive system tumors was seen both at one generation (12) and two generations (9) after cessation of high fat prenatal exposure. Because DES carcinogenicity can be transmitted by the male (13) and by blastocyst transfer (14), transplacental exposure to DES apparently affected the germ cells. In contrast, transfer of blastocysts between pregnant mice on low and high fat diets gave no indication of a germ cell effect (15). Thus, the multigenerational effect of high fat is likely to be caused by a maternal effect. A mechanism proposed to explain the carcinogenicity of dietary fat is altered sex differentiation of the brain (16). A diet high in fat increases blood levels of fatty acids, and the latter displace estrogen from steroid-binding proteins in the blood. The free estrogen is not protected from enzymatic digestion and systems dependent on estrogen, such as the developing sexually dimorphic nucleus of the fetal brain, are expected to be adversely affected (17). Consistent with this expectation, rats exposed prenatally to a high fat diet show a reduction in the size of the sexually dimorphic nucleus of the hypothalamus.[4] This could potentially alter hormone balance later in life (18) and predispose to reproductive system cancer (19). In respect to multigenerational transmission of dietary fat carcinogenesis, if the prenatally exposed animal became pregnant, it might provide an abnormal hormone environment for its developing fetus. Because differentiation of the fetus' sexually dimorphic nucleus is dependent on hormone levels (18), it might thus be altered. Conditions would then be repeated for a potentially cancer-promoting abnormality of hormone balance later in life and a predisposition to cancer of the reproductive system in these mice with only ancestral exposure to high dietary fat.

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Cancer Res 1997;57:4162-4163.