Promotional Effect of Two-Generation Exposure to a High-Fat Diet on Prostate Carcinogenesis in ACI/Seg Rats

Yasushi Kondo, Yukio Homma, Yoshio Aso, and Tadao Kakizoe

Department of Urology, Faculty of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo, 113, Japan [Y. K., Y. H., Y. A.] and National Cancer Center Hospital, 5–1–1, Tsukiji, Chuo-ku, Tokyo, 104, Japan [T. K.]

ABSTRACT

Epidemiological studies have shown an association between a high-fat diet and a high mortality rate from breast, colon, and prostate cancer. However, the promotional effect of a high-fat diet on experimental carcinogenesis has not been fully established for the prostate. In this study, the effect on prostatic carcinogenesis of two-generation exposure to a high-fat diet was investigated using ACI/Seg rats, a strain with high incidence of spontaneous prostate cancer. A high-fat diet (20% corn oil) or a low-fat diet (5% corn oil) was given to mother rats during pregnancy and the newborn male rats were fed the same diets for 60 or 100 weeks after weaning. At 100 weeks, atypical hyperplasia and adenocarcinoma of the prostate were respectively found in 73.3% (11/15) and 20.0% (3/15) of the high-fat diet group and in 20.0% (3/15) and 0% (0/15) of the low-fat diet group. There was a significant increase of atypical hyperplasia in the high-fat diet group (P < 0.05). The serum concentrations of sex hormones and the prostatic proliferative activity as measured by flow cytometry or bromodeoxyuridine labeling were not significantly affected by diet. These results showed that feeding a high-fat diet before conception and from the beginning of organogenesis had a marked promotional effect on the early stage of prostate carcinogenesis in rats.

INTRODUCTION

Since the first report on the enhancing effects of dietary fat on skin carcinogenesis in mice was published in 1930 (1), the correlation between fat intake and experimental carcinogenesis has been studied with great interest. The most firmly established fact is that high dietary intake of fatty acids enhances spontaneous and chemically induced breast cancer in rodents (2). In addition, a similar promotional effect has been reported for colon, pancreas, and lung cancer (3). The above-mentioned cancers have a common epidemiological feature, which is that their prevalence is consistently higher in Europe or North America as compared with Asia (4). The high mortality rate due to these cancers is believed to be considerably explained by the high-fat diet prevalent in Western countries (5, 6).

In this context, prostate cancer appears to be contradictory. It has the epidemiological feature of being far more common (approximately 10 times) in Europe or North America than in Asia (7). This vastly higher prevalence may reflect the interplay between various unknown and known risk factors, including age, race, a positive family history, and dietary habits (8). It is speculated, however, that epigenetic rather than genetic events have a greater influence on prostate cancer mortality. The strongest evidence for this speculation is provided by migrant studies, since the mortality rate from prostate cancer among Asian immigrants in the United States is clearly higher than in the home country, although still lower than that of American males (9, 10). For example, the age-adjusted prostate cancer incidence rate is 58.3, 31.2, and 6.3 per 100,000 for whites living in Hawaii, Japanese in Hawaii, and Japanese in Japan, respectively (7). These values indicate that prostate cancer is profoundly influenced by environmental factors, among which the dietary fat may perhaps be the most influential (11). However, except for a single study (12), various carefully conducted experiments have failed to demonstrate any association between prostate carcinogenesis and dietary fat (13–16).

One of the reasons for the lack of a promotional effect of a high-fat diet on prostate carcinogenesis in the rat might be that the special diet was started after weaning. In other words, it is possible that the high-fat diet should be started in the fetal period by feeding it to the mother animal, since maternal factors are likely to have a great influence on the organogenesis and carcinogenesis in offspring, as has been demonstrated for breast and bladder cancers (17–19). Moreover, recent studies of the Japanese have indicated that the mortality rate for prostate cancer increases with each generation after immigration (20).

The present study was carried out to examine whether a high-fat diet started in the very early stage of organogenesis had a promotional effect on prostate carcinogenesis.

MATERIALS AND METHODS

Animals. ACI/Seg rats ages 4–6 weeks were purchased from Harlan Industries, (Cumberland, IN). Each group of 4–6 rats was housed in plastic cages with woodchip bedding. The animal room was maintained at a temperature of 22 ± 2°C and a humidity of 55 ± 15%, with a 12-h light-12-h dark cycle.

Diets. The compositions of the diets used are shown in Table 1. The principal difference between the high-fat and low-fat diets was the corn oil content of 20% and 5% by weight, respectively; hence, the calorific content of the diet was 477 and 386 cal/100 g, respectively. The experimental diets were supplied every 2 months by Nippon Clea Co. (Meguro-ku, Tokyo), stored at 4°C, and replaced in the animal feeders every few days. A natural ingredient diet (CE-2) was also purchased from the same source. Food and tap water were given ad libitum. Dietary intake was measured for 4-week periods at 20, 30, 40, and 60 weeks after the commencement of the study.

Reagents and Apparatus. Bromodeoxyuridine was purchased from Sigma Chemical Co. (St. Louis, MO). An anti-bromodeoxyuridine monoclonal antibody, Dako M744 (lot no. 078), was purchased from Dako Co. (Glostrup, Denmark). An EPICS-C Flow cytometer (488-nm argon laser; Coulter Electronics Inc.) was used for flow cytometric analysis.

Experimental Protocol. Forty female ACI/Seg rats born from parents fed the natural ingredient diet were arbitrarily divided into 2 groups of 20 and fed the high-fat or low-fat diet (Fig. 1). Each of these female rats was mated arbitrarily with a male, the natural ingredient diet was fed each rat, and 60 male rats of the second generation were obtained. The newborn rats were suckled by their own mothers and then were fed the same diet as was given to their mothers. At 60 and 100 weeks after weaning, 15 rats of each group were sacrificed by an ether overdose at 3 h after the i.p. injection of bromodeoxyuridine (10 mg/kg). Blood was collected from the inferior vena cava and the prostate gland (without seminal vesicles), and the small intestine was resected.

Histological Examination. The ventral and dorsolateral lobes of the prostate were separately and fixed in 70% ethanol. For histological evaluation, two sagittal slices of the ventral lobe and two transverse slices of the dorsolateral lobe (including the urethra) were embedded in paraffin, and 5-μm sections were stained with hematoxylin and eosin. The presence of ATH and adenoma...
cancer was determined according to the criteria proposed by Bosland (21). Briefly, ATH was defined as a focal hyperplastic lesion at least 3–5 cells thick involving one or a few alveoli. The lining cells showed no or minimal pleomorphism and tended to be arranged in a cribriform pattern, but the normal alveolar architecture was not disturbed by capsular compression. Adenocarcinoma was defined as epithelial proliferation occupying 3 or more alveoli with a cribriform or solid growth pattern. The cells were clearly pleomorphic and often associated with inflammatory infiltrates. They compressed or sometimes invaded the adjacent alveoli and the normal architecture was distorted (Fig. 2).

Immunohistochemical bromodeoxyuridine staining was performed by the avidin-biotin complex method. The number of positive cells per 200 cells was counted, and expressed as the LI. The small intestine was used as the positive control.

Flow Cytometry. Nuclear suspensions were prepared from paraffin-embedded specimens according to the method of Hedley et al. (22), and the isolated nuclei were stained with propidium iodide according to the method of Vindelov et al. (23). Briefly, 40-μm-thick slices were deparaffinized, dehydrated, and incubated in 0.5% pepsin. The DI was calculated as the ratio of the channel of the G2 + M cells to that of all cells.

RESULTS

The average body weight was significantly greater in the high-fat diet group throughout the experiment (Fig. 3). Since food consumption was almost the same in both groups, this difference reflected the higher caloric intake of the high-fat diet group compared with the low-fat diet group, as confirmed four times during treatment (Fig. 4).

Minimal histological changes were found in the prostate at 60 weeks. At 100 weeks, however, the incidence of ATH was significantly higher in the high-fat diet group than in the low-fat diet group (Table 2). The lesions were generally focal and only occurred in the ventral lobe. Microscopic carcinoma was only detected in the high-fat diet group, but there was no significant difference in incidence between the two groups. No macroscopic carcinoma was found.

The LI of prostate epithelial cells was determined by bromodeoxyuridine staining (Table 3). The areas of ATH and microscopic carcinoma had a significantly higher LI than the normal cells (P < 0.05). However, there was no significant difference in LI between the high-fat and low-fat diet groups.

Flow cytometric analysis of prostate epithelial cells showed tetraploidy in all cases (Table 4). Although the DI showed no significant difference, the PI was significantly lower in the high-fat diet group than in the low-fat diet group at 100 weeks (P < 0.05). The serum testosterone level and testosterone/estradiol ratio were high in the high-fat group but were not significantly different between the high-fat and low-fat groups (P > 0.1). Serum levels of triglyceride and cholesterol also showed no differences between the two groups (Table 5).

DISCUSSION

Prostate cancer is an intriguing human malignancy. Epidemiological studies have shown that the mortality rate of prostate cancer in Asian countries is much lower than in Europe or the United States (7), although the incidence of the latent prostate cancer at autopsy is comparable (24, 25). Latent cancer may be regarded as an early stage after initiation, and certain promotional or epigenetic events are necessary for latent cancer to progress to clinically overt cancer (26).
Migrant studies have demonstrated that the incidence rate from prostate cancer increases generation by generation among Japanese-Americans after immigration (9). These facts strongly suggest that the wide differences in prostate cancer mortality may be attributable to environmental factors and a high dietary consumption of fat has often been suggested (27).

Breast and colon cancer have similar epidemiological characteristics to prostate cancer (5), and show promotion by a high-fat diet in rodent carcinogenesis models (28). In addition, Pollard and Luckert (12) have reported that a high-fat diet increases the incidence of prostate cancer and shortens the latent period in Lobund-Wistar rats treated with exogenous testosterone. In contrast to this report, however, other experimental studies on the relationship between a high-fat diet and prostate carcinogenesis have been unsuccessful in demonstrating a promotional effect on cancer induced by hormonal treatment (15), chemical treatment (29), or combined treatment (13, 14). This is also true for spontaneous prostate cancer in ACI/Seg rats (16). These inconsistencies and the generally negative relationship between a high-fat diet and prostate cancer suggest that the promotional activity of fat in rats might be too low to be detected by conventional methods if it exists at all.

A possible influence of the nutritional conditions during infancy on tumor formation later in life has been suggested by several studies. For example, rats given a high-fat diet from an early stage of life have a higher incidence of mammary tumors than those given a high-fat diet later in life (30). Sodium saccharin is nonmutagenic by itself and dose not cause carcinogenesis after one-generation continuous feeding to either conventional or analbuminemic rats, which are highly susceptible to bladder cancer (31). However, a significantly higher number of bladder tumors is induced by two-generation exposure to this agent (19). Also, in a human study high maternal estrogen levels has been shown to increase the risk of breast cancer in the children (17).

Accordingly, the present study exposed rats to a high-fat diet from a very early stage of life by feeding the mother rats the diet before conception. To detect effects solely attributable to dietary fat, we used ACI/Seg rats, which develop spontaneous prostate cancer at a high frequency without the need for carcinogens or hormones (32). In previous studies on prostate cancer using the same rat strain, no effects were detected on the incidence of either carcinoma or ATh in relation of the dietary fat level when the diets were given from 6 weeks of age onward (16).

Histological examination revealed that the incidence of prostatic ATh was considerably higher in the high-fat diet group than in the low-fat diet group (P < 0.05). Although carcinomas also tended to be more frequent in the high-fat diet group, their incidence was too low to draw a firm conclusion.

This is the first reported evidence of a facilitatory effect of dietary fat administration for two generations on experimental carcinogenesis, in the sense that starting the high-fat diet from before conception resulted in a higher incidence of ATh in the present study.

However, histochemical and flow cytometric studies showed no obvious differences between the two groups. The increased LI of the ATh lesions may have simply reflected the activation of the cell cycle, while the consistently low DI and PI values in both groups may indicate that there are generally few aneuploid or dividing cells in the prostate. Interestingly, the PI was significantly lower in the high-fat diet group (P < 0.05), which suggests that enhancement of carcinogenesis by dietary fat is not simply mediated by an increase in the proliferative activity of the prostate.

Hormonal data indicated that testosterone levels and the testosterone/estradiol ratio were increased in the high-fat group. However, these increases were not significant due to the large standard deviation.
tions, and testosterone levels or the ratio were not exceptionally high in the rats with ATH and/or cancer (data not shown). Nevertheless, there remains a possibility that high testosterone levels in the high-fat group influenced the processes of carcinogenesis. It is firmly established that testosterone plays the dominant role in prostatic carcinogenesis and that levels of this hormone are significantly higher in American blacks, the population which is most predisposed to prostate cancer (33). Exogenous testosterone administration has been shown to induce prostate cancer in several strains of rats (34) with or without a high-fat diet (12, 15). Thus, it would be naive to reject the possible role of testosterone or its metabolites in the demonstrated effect of a high-fat diet, simply because there was no statistical difference of hormone levels between the high-fat and low-fat groups.

The following limitations of this experiment should also be pointed out: (a) The diets used in this study were adjusted for fat content by altering the amount of corn oil (20% or 5%). Since the rats were fed ad libitum, the caloric intake, which may influence the general condition (35), was indeed higher in the high-fat diet group. The increase of ATH noted in our study may be attributed to this, because a high caloric intake is known to promote mammary carcinogenesis independent of fatty acid consumption (36). (b) Since the induced carcinomas were of low malignant potential compared to human prostate tumors, considerable care is required when extrapolating the present results to humans.

This study demonstrated that ingestion of a high-fat diet from before conception caused a marked increase in the occurrence of lesions believed (32) to be in an early-stage prostatic carcinogenesis. Further investigations are needed to elucidate the mechanism of this enhancement as well as to further demonstrate consistency between experimental and epidemiological observations.

REFERENCES

Promotional Effect of Two-Generation Exposure to a High-Fat Diet on Prostate Carcinogenesis in ACI/Seg Rats

Yasushi Kondo, Yukio Homma, Yoshio Aso, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/23/6129

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.