Long-Term Effects of Participation in a Randomized Trial of a Low-Fat, High-Carbohydrate Diet

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Abstract

In 1982 we started a series of pilot studies to examine the feasibility of dietary intervention with a low-fat, high-carbohydrate diet in women with extensive mammographic densities. The purpose of the present paper is to examine the long-term effects of participation in these studies by assessing nutrient intake and other variables several years after active participation had stopped.

Two hundred sixteen women were eligible for the follow-up study and were invited to attend an interview with a dietician. Data were collected by food frequency questionnaire from 157 subjects (73%), and blood was obtained from 115 subjects. Total energy intake was slightly lower in the intervention group. Total fat and percent energy from fat were significantly lower in the intervention group. The intake of all types of fat (saturated fat, linoleic acid, and oleic acid) and dietary cholesterol was lower in the intervention group; however, the polyunsaturated/saturated fat ratio did not differ between the groups. Total cholesterol and apoprotein B levels were lower in the intervention group compared to the control group. Follicle-stimulating hormone was 29% higher in postmenopausal members of the intervention group than in controls, but there was no difference in levels of estradiol. A total of 19 women enrolled in pilot studies had developed breast cancer, 5.7 times the number expected, confirming that the selection of women with extensive mammographic densities does identify a high-risk group.

These data suggest that even quite short periods of intensive dietary counselling may have prolonged effects on diet, and that once subjects have adopted new dietary habits, the habits may persist even in the absence of continued counselling.

Introduction

Breast cancer incidence and mortality are approximately five times higher in Northern Europe and North America than in Asia (1). These differences cannot be explained by inherited differences between the populations compared because immigrants from Asia to North America show a marked increase in risk, and there is a further increase in risk in the children of those immigrants (2, 3). Environmental factors must therefore exert a large influence on the incidence of breast cancer, and diet, dietary fat in particular, has long been suspected to be one of these factors.

Human ecological studies comparing breast cancer incidence or mortality with estimated dietary fat consumption of different countries show that the 5-fold variation in breast cancer rates between countries is strongly and positively correlated (r = 0.8–0.9) with international variation in estimated dietary fat intake (4). Biological effects of dietary fat on breast cancer risk can be shown in animals, including an effect on tumorigenesis that is independent of caloric intake (see Refs. 5–7 for reviews). When given with a carcinogen, dietary fat acts as a tumor promoter.

Studies in humans, either observational cohort or case control in design, have given inconsistent results (8–12). The largest cohort study conducted to date (the Nurses’ Health Study; Refs. 8 and 13) showed no association between dietary fat intake and breast cancer risk in 100,000 nurses in the USA followed for 6 or 8 years. A combined analysis of cohort studies also showed no association between fat intake and breast cancer risk, whereas a combined analysis of case control studies did show an association (14). However, the variation in the intake of dietary fat within western countries is much less than that observed between countries (4, 9). A consequence of this homogeneity is that the increase in breast cancer risk predicted from international data to exist in association with fat intake within countries is small and is further diminished by the measurement error associated with current methods of assessing dietary fat intake (4, 9).

Clinical trials in which the intake of dietary fat is reduced in an experimental group to levels lower than those common in the general population are in principle capable of overcoming the constraint imposed on observational epidemiology by the problem of homogeneity of fat intake. They are also the only means of determining whether a high-risk group of individuals can reduce its risk by changing fat intake. However, concern has been expressed about the feasibility of long-term clinical trials that involve substantial change in macronutrients such as dietary fat; in particular, there is uncertainty as to whether long-term change in dietary fat intake can be achieved (15).

In 1982 we started a series of pilot studies to examine the feasibility of dietary intervention with a low-fat, high-carbohydrate diet in women with extensive mammographic densities, a risk factor for breast cancer. The purpose of the present paper is to examine the long-term effects of participation in these pilot studies.
studies by assessing nutrient intake and other variables several years after active participation had stopped.

Subjects and Methods

Subjects. The subjects included in the present paper had earlier taken part in pilot studies carried out to assess feasibility of a randomized clinical trial to test the hypothesis that a reduction in dietary fat intake would result in a reduction in the incidence of breast cancer. A total of 295 subjects were recruited to these studies from the Breast Diagnostic Clinic of Women’s College Hospital in Toronto between 1982 and 1986. In the first pilot study, referred to as phase I, 230 women were recruited between 1982 and 1984 and followed for 1 year. In the second pilot study, referred to as phase II, 65 women were recruited between 1984 and 1986 and followed for 2 years. In addition, 21 women from phase I continued into phase II.

The same criteria of eligibility applied to phases I and II. Women were eligible for each of the pilot studies if they were ages 30–65 years and had a mammogram indicating that at least 50% of the breast volume was occupied by radiographic density. Women were excluded from the pilot studies if they had been diagnosed previously with cancer, were on medically prescribed diets, or were pregnant or breast-feeding.

Women who were eligible and willing to participate were assigned randomly to one of two groups. One group received a dietary intervention designed to reduce intake of fat, and the other, a control group, was not counselled to change their diets. Further details of these studies have been reported previously (16–18), and the nature of the dietary intervention is described only briefly in the next section.

Dietary Intervention in the Pilot Studies. Subjects randomly allocated to the intervention group were counselled to reduce their dietary intake of total fat to a target level of 15% of energy. An individualized diet plan, using a food exchange system, was designed for each subject based on a detailed assessment of dietary habits by diet history at entry into the study. In the diet plan, fat was replaced by the isocaloric exchange of carbohydrate. Each participant received a dietary scale and a handbook containing her prescribed diet pattern, product information, restaurant guides, and more than 100 tested low-fat recipes. After randomization, subjects in the intervention group were seen once every month for 12 months and those in the control group once every 4 months for 12 months in both pilot studies. In phase II, subjects in both groups were seen, in addition, twice during the second year.

At each visit, subjects in both groups provided records of all foods and beverages consumed on three consecutive days. The food records were used to provide feedback to subjects regarding compliance with the dietary prescription. Particular attention was paid to the maintenance of caloric intake and the preservation of initial body weight at the clinic visits. Food records were subsequently analyzed by the Nutrition Coordinating Center, University of Minnesota, Minneapolis, using the Nutrient Data System.

Recruitment of Subjects for the Follow-up Study. Each year since the completion of the pilot studies, a demographic questionnaire had been mailed to each participant to obtain information about various health outcomes, including breast cancer and other breast disease. Of the 295 women who entered the phase I and II pilot studies, 79 were not contacted for the follow-up study for the following reasons: 17 women had been diagnosed with breast cancer during the pilot studies or since the studies had ended; 3 had died; 1 had achieved a secondary end point (kidney cancer); 4 had been lost to contact by our staff; and 54 had dropped out of the pilot studies. (A dropout in this context refers to a subject who severed contact with a pilot study after randomization.) Therefore, 216 women were considered eligible and were sent letters inviting them to participate in the follow-up study by attending a personal interview with a dietitian. Women who could not attend a personal interview or lived outside the province of Ontario were asked to complete a short demographic questionnaire by mail or telephone.

Data Collection for the Follow-up Study. Data were collected for the follow-up study reported here between June 1992 and February 1993. Body weight was measured to the nearest 0.1 kg on a balance scale with shoes removed, and height was measured using a stadiometer.

A semiquantitative FFQ was used to estimate average daily intake of energy, fat, and other nutrients over the previous year. The food questionnaire (Health Habits and History Questionnaire, computer scannable, January 1992 version), nutrient data base, and computer software program for analysis of the questionnaire were modifications of the instruments developed by Block et al. (19; National Cancer Institute). The FFQ included questions about the portion size and frequency of intake of approximately 100 foods.

The demographic questionnaire was used to obtain updated information on the occurrence of cancer, other illnesses, breast biopsies and symptoms, mammograms, medications, smoking, physical activity, and menstrual cycle/ovulatory factors. Physical activity was measured on a seven-category scale ranging from no deliberate exercise to heavy exercise on a regular basis.

Subjects were asked to provide a nonfasting serum sample at the clinic visit. Fresh serum samples were sent on ice to the Lipid Research Laboratory at St. Michael’s Hospital in Toronto. Total cholesterol was measured on fresh serum and HDL-C, apo A-I, and apo B using frozen serum. Cholesterol was measured using the Technicon RA1000 and Technicon enzymatic reagents (Technicon method SM4–0139G86). HDL-C was measured as the cholesterol in the supernatant after precipitation of the non-HDL cholesterol using heparin and 46 mM magnesium chloride with removal of the magnesium ion with sodium bicarbonate before measurement of cholesterol. Apo A-I and apo B were measured using a Behring BN100 nephelometer and Behring reagents calibrated according to the manufacturer’s instructions. Assays of FSH and estradiol were performed by the Steroid Laboratory of the Wellesley Hospital, Toronto by RIA.

Variables of interest collected at baseline and during the pilot studies were also used in the analyses. These variables included demographic characteristics, height, weight, and nutrient intakes.

Statistical Analysis. Data are presented as means and SDs. Variables were examined for normality, and those with skewed distributions were log transformed. Differences between the intervention and control groups at various time points were examined using Student’s t test for continuous variables and with χ² for categorical variables. In instances in which log transformation did not normalize the distribution of a continuous variable, the Wilcoxon rank sum test was used for group comparisons. The Pearson and Spearman correlation coeffi-
Results

Of 216 eligible women, 159 (74%), completed the full demographic questionnaire, and 157 (73%) completed, in addition, the FFQ. Twenty-two of the FFQs were self-administered and were returned by mail. Blood serum samples were collected from 115 of the women who attended clinic appointments. The 159 subjects who responded to our invitation to take part in this follow-up study were compared to the 59 who did not according to demographic characteristics, the time they were in pilot studies, and the time elapsed since they left these studies, as well as according to intake of nutrients at baseline and during the studies. No statistically significant differences were found in these variables between responders and nonresponders.

Among reasons for nonresponse, three women were unwilling to respond to the follow-up study and two could not be contacted. Two women had developed breast cancer since the last contact, and data were not collected from them.

Characteristics of Subjects. Table 1 shows selected demographic characteristics of the subjects who completed the FFQ according to the group to which they had been randomly assigned during the pilot studies. On average, the subjects had spent 1.4 years (SD, 0.5) participating in pilot studies and were evaluated in the follow-up study a mean of 7.8 years (SD, 1.8) after participation had ended. The members of the intervention and control groups were similar in terms of age, weight, body mass index, physical activity score, years of education, and marital status. The length of time that they took part in pilot studies and the length of time since they had completed their active participation in these studies were also similar.

Nutrient Intake. Table 2 shows the results of the FFQ for intake of selected nutrients in the follow-up study. Total energy intake was slightly lower in the intervention group. Total fat and percent energy from fat were significantly lower in the intervention group. The intake of all types of fat (saturated fat, linoleic acid, and oleic acid) and dietary cholesterol was lower in the intervention group; however, the polyunsaturated (linoleic acid)/saturated fatty acid ratio did not differ between the groups. Protein intake was similar in the two study groups, and percent energy from carbohydrate (but not total carbohydrate grams) was slightly greater in the intervention group. Intake of calcium, total dietary fiber, β-carotene, and alcohol did not differ between the groups.

Biochemical Variables. Table 3 shows the serum total cholesterol, HDL-C, apoprotein, and hormone assay results obtained from the 115 subjects who were willing to give blood. Because of the limited number of subjects and the greater variability of hormone assays in premenopausal women, we performed hormone assays only on subjects who were postmenopausal at the time of follow-up.

Total cholesterol and apo B levels were lower in the intervention group compared to the control group; however, these differences did not reach statistical significance (P = 0.13 and P = 0.07, respectively). HDL-C and apo A-1 levels were not different between the two groups.

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Long-Term Effects of a Dietary Intervention Trial

FSH was 29% higher in the intervention group than in controls, but there was no significant difference in levels of estradiol, either in hormone users or nonusers. The difference in estradiol levels was statistically significant after controlling for weight \((P = 0.04)\), and was observed only in subjects not taking exogenous hormones in whom the mean values for FSH were 128 IU/liter and 96 IU/liter in intervention and control groups, respectively \((P = 0.03)\).

### Comparison of Nutrient and Serum Cholesterol Values and Weight during Pilot Studies and at Follow-up

Energy intake, percentage energy from fat, weight, and serum cholesterol values during pilot study participation and during the follow-up study are shown in Table 4. No differences were observed between the intervention and control groups in FSH levels. However, the serum cholesterol level on follow-up remained lower in the intervention than in the control group, although the difference was only marginally significant \((P = 0.09; \text{Table 4})\).

**Table 4.** Energy intake, percentage energy from fat, weight and serum cholesterol at baseline, during pilot studies and at time of follow-up (data presented are for a sample of women with dietary data during pilot study and follow-up study).

<table>
<thead>
<tr>
<th></th>
<th>INTERVENTION</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>Baseline (^b)</td>
<td>1717 (366)</td>
<td>36.9 (6.7)</td>
</tr>
<tr>
<td>Pilot study (^d)</td>
<td>1531 (305)</td>
<td>33.9 (5.3)</td>
</tr>
<tr>
<td>Follow-up (^e)</td>
<td>1460 (376)</td>
<td>31.7 (7.3)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>52</td>
<td>4.95 (1.04)</td>
</tr>
<tr>
<td>Weight (kg) (^f)</td>
<td>60.9 (7.5)</td>
<td>4.89 (1.01)</td>
</tr>
<tr>
<td>Total energy (kcal)</td>
<td>1791 (374)</td>
<td>1738 (330)</td>
</tr>
<tr>
<td>% Energy from fat</td>
<td>36.9 (6.3)</td>
<td>35.6 (4.5)</td>
</tr>
<tr>
<td>FFQ</td>
<td>1573 (365)</td>
<td>1573 (365)</td>
</tr>
<tr>
<td>% Energy from fat</td>
<td>35.2 (5.6)</td>
<td>35.2 (5.6)</td>
</tr>
<tr>
<td>Weight (kg) (^g)</td>
<td>60.1 (8.3)</td>
<td>60.4 (8.4)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>59.6 (7.3)</td>
<td>59.6 (7.3)</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>4.65 (0.86)</td>
<td>4.98 (0.89)</td>
</tr>
<tr>
<td>Mean of all food records during study participation, excluding baseline for intervention group. (^a)</td>
<td>64.9 (11.2)</td>
<td></td>
</tr>
<tr>
<td>Mean of 3 nonconsecutive-day food records before randomization. (^a)</td>
<td>5.13 (0.85)</td>
<td></td>
</tr>
<tr>
<td>Mean (SD). (^a)</td>
<td>5.7 (95% confidence interval = 3.63–8.92)</td>
<td></td>
</tr>
<tr>
<td>FFQ</td>
<td>60.0 (8.2)</td>
<td>60.4 (8.4)</td>
</tr>
<tr>
<td>% Energy from fat</td>
<td>6.3 (0.9)</td>
<td>5.40 (0.81)</td>
</tr>
<tr>
<td>Weight (kg) (^h)</td>
<td>59.6 (7.3)</td>
<td>63.9 (9.7)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>60.1 (8.3)</td>
<td>60.1 (8.3)</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>4.89 (1.01)</td>
<td>4.98 (0.89)</td>
</tr>
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<td>Mean (SD). (^a)</td>
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<tr>
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<td>60.1 (8.3)</td>
<td>60.1 (8.3)</td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>4.98 (0.89)</td>
<td>4.98 (0.89)</td>
</tr>
<tr>
<td>Mean (SD). (^a)</td>
<td>5.7 (95% confidence interval = 3.63–8.92)</td>
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<td>63.9 (9.7)</td>
</tr>
</tbody>
</table>

\(^a\) Variable log transformed for \(t\) test.
\(^b\) Mean of 3 nonconsecutive-day food records before randomization.
\(^c\) Mean (SD).
\(^d\) Mean of all food records during study participation, excluding baseline for intervention group.
\(^e\) FFQ.
\(^f\) \(P \leq 0.0001\). (All \(P\) values are for comparison between intervention and control groups.)
\(^g\) \(P < 0.05\).
\(^h\) FFQ.
\(^i\) \(P = 0.06\).
\(^j\) \(P = 0.001\).

**Discussion**

The data shown in this paper were obtained from the observation of women who had taken part in pilot studies almost 8 years earlier. These studies had been designed to assess the feasibility of a randomized controlled trial to examine the effect of dietary change on the incidence of breast cancer and were necessarily of relatively short duration, lasting 12 to 24 months. The results shown here provide information about the feasibility of cancer prevention trials of longer duration using dietary intervention. This information concerns the long-term effects of dietary counselling, the risk of breast cancer in the subjects...
selected, and the finding of hormonal differences between former members of intervention and control groups that may be relevant to breast cancer risk.

Information about current dietary intake was obtained from subjects taking part in the present study using a FFQ an average of 7.7 years after the subjects had finished participating in pilot studies. Current data on intake show statistically significant differences in intake of total calories, fat, and carbohydrates between former members of intervention and control groups. The intake of total calories and percent energy from fat are lower, and intake of percent energy from carbohydrate is higher, in former members of the intervention group. Similar results have been described in the follow-up of participants in the Women's Health Trial who, an average of 1 year after the cessation of dietary counselling, continued to maintain a substantially reduced-fat diet (20).

These data must be interpreted in light of the limited available information about the validity of dietary information. The validity of dietary information collected during the trial is supported by data on changes in serum cholesterol and by the analysis of duplicate meals (18). We found a close quantitative relationship between the changes observed in serum cholesterol and the changes predicted from changes in the intake of saturated and polyunsaturated fats using the Key formula (18). We also found close correspondence between intakes recorded in food records and those analyzed chemically in duplicates of food consumed (18). We have also assessed the accuracy of estimates of usual energy intake derived from food records in a subset of 29 women taking part in the trial. Reported energy intake was derived from 7-consecutive-day food records, and total energy expenditure was measured by the doubly labeled water method over a 13-day period. We found that food records underestimated energy requirements by about 20%, and that the adequacy of reporting was similar between the control and the intervention groups (82.1% and 77.8%, respectively; P = 0.55). This measurement error in reported energy intake is similar to that found by others and does not invalidate the group comparisons that are the main application of the collected food records.

The reported differences in dietary fat intake at follow-up are supported by the tendency toward lower serum cholesterol and apo B levels in the intervention group members as compared to the control group. However, the FFQ may be less sensitive than food records for low-fat diets because of limited information on fat-modified foods and recipes. White et al. (20) compared a similar FFQ to food record data collected at the same time points and observed that the FFQ overestimated percentage fat intake by 2.4% in the intervention group of the Women's Health Trial. Therefore, the percentage fat intake in the intervention group women in this follow-up study may be lower than reported on the FFQ.

Because not all eligible subjects attended the follow-up clinic at which data were collected, the differences observed between members of the control and intervention groups who did attend may not be representative of the groups as a whole. Nevertheless, a relatively high response rate (74%) was obtained, and the results apply to a substantial proportion of those who participated in the pilot studies and were eligible for the follow-up study.

A major question about the feasibility of long-term dietary intervention trials directed at changing cancer incidence concerns long-term dietary compliance. The findings presented here suggest that even quite short periods of intensive dietary counselling may have prolonged effects on dietary intake and that once subjects have adopted new dietary habits, these habits may persist even in the absence of continued counselling. It is expected, therefore, that continued counselling will achieve differences in intake between intervention and control groups at least as large and presumably larger than those seen here. The 7.7 years that elapsed between the end of dietary counselling and the present follow-up study are about 80% of the projected 10-year duration of the dietary intervention cancer prevention trials that are now in progress.

A further aspect of the feasibility of cancer prevention trials addressed by the present data is the number of cancers that can be expected to develop during the period of observation. The subjects studied here had been selected because they all had extensive tissue densities on mammogram, a risk factor for breast cancer (21). Follow-up confirms that the group selected is, 8 years later, still at substantially increased risk of breast cancer. There is a small excess of cancers among members of the control group that is not statistically significant.

In the present study we also found biochemical differences between intervention and control subjects that are of possible relevance to breast cancer risk. Among postmenopausal subjects, FSH levels were significantly higher in intervention subjects compared to the control subjects. This difference in FSH was not accompanied by any difference in concentrations of total estradiol. However, lower levels of free estradiol or higher levels of sex hormone-binding globulin in the intervention group, which were not measured in the present study, might explain the observed findings. Alternatively, differences in enterohepatic circulation of estradiol, as have been described in vegetarians and with increased intake of dietary fiber (22–27), might explain higher levels of FSH. In either case, the present findings suggest long-term effects of dietary change on ovarian function. Such an effect is likely to be relevant to breast carcinogenesis in light of the abundant epidemiological and other data that suggest that ovarian function is related to risk of breast cancer (28–31).

References
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Long-term effects of participation in a randomized trial of a low-fat, high-carbohydrate diet.
