

A Prospective Study of the Intake of Vitamins C, E and A and the Risk of Breast Cancer.

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Introduction

Many of the common cancers have vague aetiological influences that can be seen to operate when large populations change their environmental circumstances (e.g., gastric cancer in Japanese). These aetiological influences have been hypothesized as viral, environmental and dietary.

Basic science research has clarified the roles of some vitamins. Antioxidation and cell differentiation are two such roles for vitamins C, E and A. Before vitamin manipulation can be utilized as a carcinogenesis-modifying manoeuvre, a difference in consumption must be shown to result in an altered cancer incidence.

Such evidence can be gathered from a prospective randomized trial, however such a trial would take a very long time, and because of sample size considerations may be incapable of determining a positive result. One prospective trial of antioxidant vitamin (C, E) intake on colonic adenomata has recently been reported⁵⁵ and identified that the above problems may have prevented a positive result being detected. A cohort study, with retrospective or prospective determination of exposure to vitamins, could be used to predict the required sample size for further studies.

Such cohorts have been previously identified for the purpose of other studies (e.g., the Framingham study, Nurses Health Study) and have established methods to determine both exposures and outcomes.

This is the second cohort study to be analysed in this assignment, only issues not previously discussed will be enlarged upon.

Summary

This report describes the results of a study that included data permitting the correlation of dietary intake and the subsequent development of breast cancer.

'Trial' Design

This is a prospective cohort study classifying patients on the basis of exposure at or near the time of exposure. The cases are provided by the Nurses' Health Study (NHS)⁵⁰. The study consisted of a baseline questionnaire (demographics, reproductive/medical history, lifestyle choices) and second yearly questionnaires on outcomes (mainly cancers and cardiovascular disease), exposures (as per baseline questionnaire) and new variables of interest.

Study Aim

The author's aim was to quantify the risks of breast cancer development that result from different levels of consumption of vitamins (specifically C, E and A).

Sample size

Statistical Power

The cohort study is useful for delineating the effects of a common exposure where the disease outcome dependent on exposure is also common; for example, the NHS has been used for assessing links between hormone variations and cardiovascular disease⁵¹.

'Trial' Conduct

Eligible Population

The eligible population were self-selected in 1976 when invited to participate in the NHS. This large cohort study enrolled 120,000 nurses by mail survey. The nurses were married females aged 30-55 years and resident in 11 states of the USA. The total pool of nurses, and the number refusing to be enrolled are unknown. This study demonstrates restriction of participants as a method for reducing the likelihood of confounding variables (sex, occupation and extremes of age are not variables).

Patient Selection - Unexposed

Since vitamins A, C and E are present in food, and are necessary for normal functioning, there can be no unexposed group.

Patient Selection - Exposed

Exposure in this study consisted of a dose estimation of vitamin intake by subdividing intake into quintiles. The rationale for the boundary lines are not described. The comparability of these quintile groups, in terms of race or age, was not addressed.

Data Sources

The enrolled population was surveyed for food type, amount and frequency intake biennially. The 1980 survey included a semi-quantitative 61 item scale, which was expanded to 121 items in the 1984 survey. It also included information on vitamin supplements used. The last survey included in this analysis occurred in 1988. Derivation of vitamin values utilized the USA Department of Agriculture energy content and vitamin levels for foods, and a compiled database of vitamin content in supplements.

The availability of outcome data rested mainly on patient reports. Breast cancer data was derived from pathology and hospital records after patients reported "breast cancer". Patients were very accurate with their breast cancer reports (97% of reports were verified, 99+% were accurate), and as a result patients were considered as breast cancer cases when confirmation could not be obtained. There are no reports of searches of tumour registries to confirm non-reported cases.

Patient Exclusion

No patient exclusion was reported, however it appears that only data that was available on patients who completed two surveys was used. The intake described in the survey was assumed to be the intake for the entire survey period, accumulating 'person-months without cancer' to the quintile of intake.

Treatment Description

There was no therapy described. The study looks only at breast cancer incidence.

Pathology

Given the patient numbers, pathological verification of the diagnosis of breast cancer was excellent. The veracity of the patient-reported diagnosis was upheld. Unfortunately, no attempt was made to quantify the number of patient-unreported breast cancers via tumour registries, or death certificates.

Trial Analysis

Statistical Analysis

The vitamin intake levels were subdivided into quintiles based on dietary intake, with and without adjustment for energy intake. The choices of boundaries and number of groups are not discussed. Recommended daily consumption for the general population are not indicated, nor is the intake distribution compared to population rates. The use (both dose and duration) of supplements further subdivided these groups.

The recommended daily intake (RDI) of the vitamins studied, for the USA and Australia⁵¹, are listed below. Average daily intakes were not obtainable. It is interesting to note that Vitamin C and A levels are well above the RDI, while the Vitamin E levels represent less than the RDI. In fact the three lower quintiles of vitamin E intake represent sub-maximal intake.

VITAMIN INTAKE		RDI (USA)	RDI (Australia)	Study range (min. max boundaries)
Vitamin C	(mg/day)	60	30	<93 >359
Vitamin A	(IU/day) [retinol equivalents]	2664	2475	<6630 >17640
Vitamin E	(IU/day)	8	7	<3.9 >24.1

Incidence rates were determined by patient reports (cases - with later attempted pathologic confirmation) and by totalling person-months without breast cancer. This occurred for each assigned quintile intake of vitamin. The month of diagnosis, menopause or death was taken as the terminating time for accrual of person-months to each group. This allowed the calculation of incidence (crude and age-adjusted) of breast cancer in each quintile. The lowest quintile exposure was chosen as the reference group and used in the calculation of relative risk. Relative risks were presented with 95% confidence intervals with two-tailed p values.

The proportional hazards analysis is a statistical method utilized to identify confounding variables, and is specifically utilized when there are differing observation times within the groups. A proportional-hazards analysis was conducted looking for the influence of age, menopausal status and other variables. The analysis was updated each two years for altered variables (i.e., at each biennial survey, another portion of the participants would have entered menopause).

The analysis was conducted after the identified outcome had occurred, predetermined as well as retrospectively determined variables can be included. The analysis was then used to identify independent variables.

Unfortunately where two independent variables are found, codependence or the presence of a confounding variable cannot be ascertained. The results of such an analysis should be only regarded as a pointer directing further investigation where stratification of the "significant" variables occurs.

There are several statistical problems evident in this study :

- a. The vitamin intake for 1980/1984 was correlated with breast cancer incidence up to 1988. The survey period covers only 8 years, a short portion of the probable carcinogenic time needed for the development of invasive breast cancer.
- b. it appears that participants only needed to return two surveys to be eligible for analysis. The extrapolation of this data is problematic :
 - i. the assumption that vitamin intake described in the 1980 and 1988 surveys gives a meaningful view of vitamin intake for 8 years is unsupported and counterintuitive, and if implied requires verification.
 - ii. the assumption that vitamin intake described in a biennial survey is valid for the entire inter-survey period, even if provided as requested, has not been proven. Correlation coefficients of 0.35 - 0.75 for the survey instrument were verified on only a four week diary.
- c. the survey is acknowledged as a "semi-quantitative food-frequency questionnaire". Given that this semiquantitative survey was combined with assumptions that vitamin content approximated the USA averages, the inaccuracy involved with quantitation of vitamin intake must be large; some attempt to quantitate this error should be included.
- d. quantitation of the variation in vitamin intake during the biennial survey should have been undertaken.
- e. although person-months for a survey quintile were only accrued until the next survey (e.g., 24 person-months/person assigned to 1980 quintiles until the 1982 survey was completed), the authors do not disclosed the method of assigning person-months derived from the 1988 data.
- f. energy-adjusted values were undertaken on the basis of 1980 and 1984 surveys only. The data obtained at each biennium would allow calculation of energy-adjusted values for that period.

Confounding Variables

1. vitamins, except as supplements, are derived from foods. The possibility exists that the type of food may be protective independent of the vitamin content, i.e., that vitamin levels may be a marker for the protective agent.
2. the number of unreported breast cancers. Preinvasive lesions (DCIS, LCIS) may be underreported since they may not be described as "cancer" to the patient.
3. the recall effect on vitamin intake after the diagnosis of breast cancer. This bias is only likely to affect the survey after development of breast cancer.
4. actual vitamin intake levels are unknown. Extrapolation from semiquantitative data is inaccurate. In addition, the sporadic use of vitamins is not compared with their consistent use.
5. the effect of quantifying vitamin intake on subsequent consumption of vitamins. The survey may combine with media information to promote vitamin use, or make participants more health aware.
6. the possibility that both vitamin intake and a high risk of breast cancer are linked through an unidentified/unmeasured covariable such as lifestyle.
 - i. a glimpse of this possibility is evident in the subset analysis showing an increased frequency of mammography in the quintiles with higher vitamin intake (preformed vitamin A, carotenoid).
 - ii. societal trends have occurred during the 1980-1988 study period in food consumption, drug intake and exercise levels. The reduced use of processed foods, and increased intake of vegetables may lead to a higher vitamin intake as well as reduced breast cancer incidence.
7. vitamin intake may affect the rate of progression of established breast cancers, rather than the incidence of breast carcinogenesis, i.e., increased lead-time bias.
8. the exclusion of DCIS, a premalignant lesion associated with a 10% invasive carcinoma incidence at 5 years when completely excised (see study 1), is not warranted. The treatment of DCIS may substantially alter a woman's risk of later cancer (cure in the case of mastectomy, 10% cancer after lumpectomy). The finding of only 127 cases of DCIS in the period 1980-1988 when screening mammography is less than expected. A quarter of mammographically-detected lesions are DCIS, and this population is more likely than others to be screened.
9. the analysis uses the same cases to investigate the effect of intake level. The interrelationship of intake excesses and deficiencies are not addressed. This study looked at 5 vitamin groups in two analyses, such investigations of multiple subgroups within the same population have a higher probability of finding a "significant value".

Trial Outcome

Criteria for Evaluation

Endpoints

Patients were analysed at development of breast cancer, death and the dates of each.

Bias

Classification of vitamin intake was largely independent of outcome (development of breast cancer).

The study group is restricted in an attempt to eliminate potential confounding variables (socioeconomic status, sex, age, educational status).

Loss to follow-up

There are no reports apart from survey return rates to survey to indicate that loss had occurred. Most of the deaths were identified.

Non-participants

Non-participants did not reply to surveys.

Results

The analysis showed that :

1. In this population group, Vitamin C and A intakes are well in excess of the RDI, while approximately 60% are vitamin E deficient.
2. Lower Vitamin C intake is not associated with an increased risk of breast cancer, and higher intake is not protective for breast cancer.
3. Lower Vitamin E intake is not associated with an increased risk of breast cancer, and higher intake is not protective for breast cancer. An inverse trend for breast cancer risk was removed when vitamin A was included in the multivariate analysis.
4. Higher Vitamin A intake is associated with a reduced risk of breast cancer, especially in the premenopausal group, and in the group attending for mammography.
5. The carotenoids are less active than other sources of food or supplemental Vitamin A.
6. Higher Vitamin A levels combined with higher vitamin C and E levels were associated with even more reduced risk of breast cancer.
7. Supplemental vitamins are not protective for breast cancer, except perhaps in the group who have poor dietary vitamin A intake.
8. Higher vegetable intakes are associated with reduced risk of breast cancer.

Conclusion

The authors conclude that higher vitamin A levels are associated with reduced breast cancer, and that the association is small, but not confounded by risk factor or screening distribution. They identify a consistent non-linear relationship with vitamin intake. The usefulness of vitamin A supplements, only when diet is deficient, is demonstrated.

The authors point to previous literature, and the action of vitamin A to provide a biological basis for their findings. They recommend that a randomized trial of vitamin A supplements should be undertaken.

OVERVIEW

The authors of this study are to be commended for their meticulous followup of a very large cohort. Unfortunately, this report has not served to clarify the relationship of vitamins to breast cancer.

Confounding variables that may operate in the formation of breast cancer have been well accounted for, but confounding variables that relate to food and vitamin supplement intake have not. The use of biennial surveys to obtain data is fraught with uncertainty when extrapolating point-in-time data to the intervening period.

The statistical analysis is not clearly defined with respect to choices of quintile boundary, and involves multiple variables (at least 18 were included). The use of vitamin intakes far in excess of the RDI are compared to intakes that are less than the RDI level.

There are three trends that have emerged from the data - vegetables and food-derived vitamin A are "protective", while Vitamin A supplements are not "protective" unless a state of dietary deficiency already exists. Paradoxically the authors then suggest a randomized trial of vitamin A supplements! A trial where diets were randomized to adequate vegetable-derived vitamin A or adequate non-vegetable derived vitamin A would have more merit in determining the aetiological role of vitamins and the food from which they are derived.