

Clinical Metabolic Studies in Cancer Research¹

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Clinical metabolic studies are being used increasingly to study the role of nutrition in cancer etiology and prevention. These studies have important applications in at least five areas. The kinetics and toxicity of potential chemoprevention agents can be investigated in preparation for intervention studies. Nutrient levels proposed as compliance markers in intervention studies can be assessed under rigorous control. Potential mechanisms of action of nutrients can be evaluated. And intermediate endpoints, markers of biologic damage, can be measured before and after controlled dietary manipulations. As a result of these contributions, clinical metabolic studies are taking on a new and important role in the interdisciplinary approach to cancer research. © 1989 Academic Press, Inc.

INTRODUCTION

Clinical metabolic studies evaluate some element of the metabolism of factors thought to influence disease risk in humans. These studies are potentially an important component of diet and cancer research, for both our understanding of cancer etiology and our efforts in cancer control. In clinical metabolic studies, human nutrition or nutrition-related parameters are assessed, using either biochemical or morphologic measurements as endpoints. These studies evaluate differences between individuals or changes in the same individual, in response to a carefully defined nutritional change.

Clinical metabolic studies can be categorized into two broad types: observational studies and interventions. Observational studies can, in turn, be either cross-sectional or longitudinal in nature.

The optimal setting for a clinical metabolic study is dictated by the degree of control and the rigor of sampling required. Possible settings range from field studies among free-living subjects to closely monitored interventions in hospital metabolic units.

This article describes and illustrates some possible applications of clinical metabolic studies in cancer research. There are five broad areas of cancer research in which clinical metabolic studies can be applied. These areas are kinetics, toxicity, markers, mechanisms, and intermediate endpoints. Each of these areas is discussed in detail below with illustrations drawn from recently completed investigations.

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KINETICS

The basic rationale for conducting kinetic studies in the setting of nutrition and cancer is to learn about the absorption, excretion, and retention of potential cancer preventive substances, be they micronutrients or synthetic agents; predict time to steady state and estimate total body load; and help identify those body pools that can be monitored (1). Absorption and excretion information is desirable before a substance is given as part of an intervention plan. Similarly, estimates of time to steady state and total body load can be useful in optimizing dose intervals and predicting toxicities. Etiologic studies evaluating the relation of a substance to a specific cancer are most informative when the level of the substance is measured in the body pool most relevant to the cancer under study.

An example of a clinical metabolic study of this type is a pharmacokinetic pilot study of selenium conducted as part of the collaboration between the United States Department of Agriculture (USDA), and the National Cancer Institute (NCI) to study nutrition and cancer (2). Selenium, a trace element that has been shown to inhibit the development of a number of different tumors in animals and has been associated with a reduced risk of cancer in several epidemiologic studies, is currently being tested as a chemopreventive agent in several clinical trials in humans.

In this study, six normal subjects were fed a controlled diet containing a constant amount of selenium (90 $\mu\text{g}/\text{day}$) for 15 days. On the third day of the study, a single labeled oral dose of 200 μg of sodium selenite ($^{74}\text{SeO}_3$), an inorganic form of selenium, was given. Following the dose, multiple blood, urine, and fecal samples were collected. Selenium concentration was measured in each of the samples by isotope dilution using gas chromatography-mass spectrometry.

The degree of the complexity of the kinetics of sodium selenite was not initially appreciated. Attempts to formulate simple models that simultaneously satisfied all the experimental data proved unsuccessful. Using these data, a complex compartmental model describing the kinetics of selenite metabolism is being developed. Following the development of an adequate model, data from a larger pharmacokinetics study will be used in an attempt to refine the selenite model and to develop a model for the metabolism of selenomethionine, an organic form of selenium. The different compartments in such a model may represent different forms of selenium and these, in turn, may have different implications for toxicity and cancer prevention.

Another example of a kinetics study which is substantially simpler than the selenium kinetics modeling approach is the comparison of plasma responses to single ingestions of selected vegetables high in carotenoids (3).

TOXICITY

The primary purpose of conducting clinical metabolic studies of toxicity is to determine the maximum safe dose of a potential chemopreventive agent and to monitor possible side effects. This is the same purpose for which toxicity studies are conducted on agents intended for use in cancer treatment. The principle generally assumed is that the dose-response relation between a chemopreventive

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agent and cancer prevention is linear. This means that, short of toxicity, more is always better, so we would like to know the highest nontoxic dose that can be given. Knowledge of potential toxicity in this setting is particularly important because, unlike cancer treatment trials where participants all have cancer and toxic therapies are acceptable, participants in chemoprevention trials are generally disease-free at entry into the study although they may be at high risk for cancer. The level of risk may determine the acceptable level of toxicity.

A number of epidemiologic studies have associated consumption of foods high in carotenoids with a lower risk for selected cancers. Several prevention trials are currently testing the hypothesis that β -carotene ingestion reduces cancer risk (4, 5). A study conducted as part of the USDA-NCI collaboration and reported by Micozzi *et al.* (6) illustrates how one aspect of β -carotene toxicity, carotenoderma, was evaluated.

In this study, the relation between plasma levels of carotenoids and carotenoderma was evaluated in 30 healthy men receiving carotenoid supplementation for 42 days. Five subjects were randomly assigned to each of six treatment groups differing in type and amount of total carotenoids. The 30-mg carotenoid groups received either 30 mg/day β -carotene capsules or 30 mg/day of total carotenoids from carrots. The 12-mg carotenoid groups received either 12 mg/day β -carotene capsules or 12 mg/day of total carotenoids from tomato juice. The 6-mg carotenoid group received 6 mg/day total carotenoids from broccoli. A control group received placebo pills. Apart from the supplements, all participants consumed controlled diets containing <1.5 mg/day total carotenoids.

Results from this study indicate that definite carotenoderma was observed only in the five subjects who took 30 mg/day β -carotene capsules. Figure 1 shows the relation of plasma levels to carotenoderma in these subjects over the course of the study. The figure demonstrates that carotenoderma typically was first observed after plasma levels had peaked and begun to fall. Carotenoderma was first observed between 3½ and 7 weeks after starting supplementation and persisted for 2–7 weeks after supplementation ended. Despite uniform levels of intake, peak plasma levels ranged from 416–603 $\mu\text{g}/\text{dl}$ (median = 525 $\mu\text{g}/\text{dl}$) in subjects with carotenoderma, while levels in subjects with no skin yellowing ranged from 115–305 $\mu\text{g}/\text{dl}$ (median = 133 $\mu\text{g}/\text{dl}$). No other physical or biochemical abnormalities were observed in these subjects.

Another illustration of the use of clinical metabolic studies in evaluating toxicity of potential chemopreventive agents is an assessment of the potential toxicity of selenium conducted among residents of South Dakota where blood levels of selenium are among the highest found in the United States (7).

MARKERS

Biochemical or other markers that reliably reflect specific dietary changes can be used to monitor compliance in intervention studies. In addition, markers can be used to compare different measurements that attempt to estimate the same nutritional parameter, and to validate questionnaires for use in other settings. Markers may also be found that are capable of predicting disease outcome and may thus serve as intermediate endpoints. A study of the relation of changes in various meta-

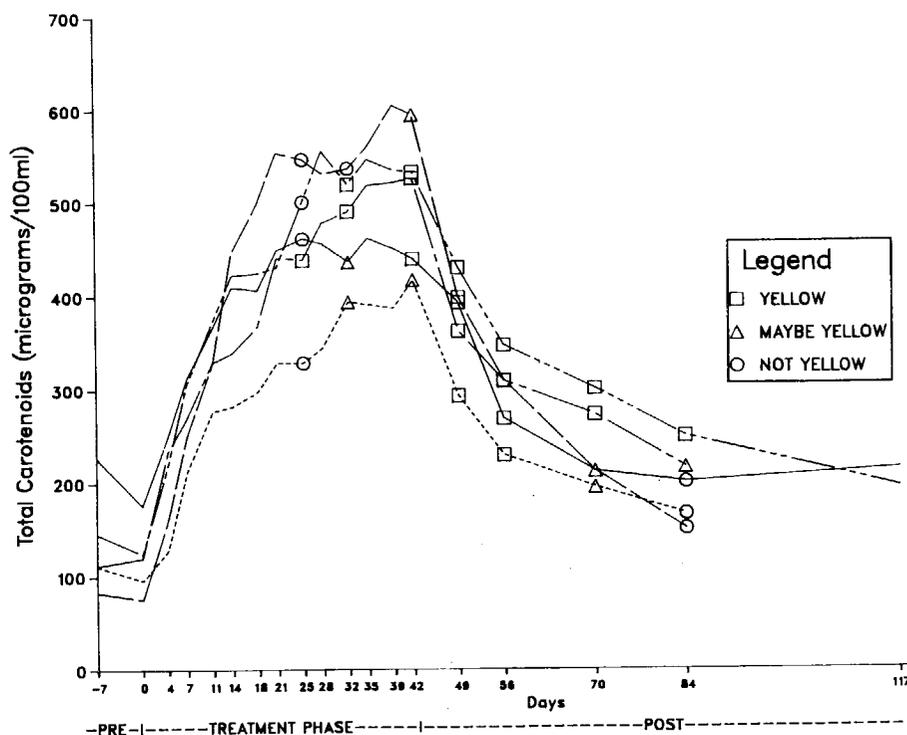


FIG. 1. Total plasma carotenoid levels and carotenodermia in 5 subjects receiving 30 mg β -carotene capsules daily (used by permission, *Amer. J. Clin. Nutr.*, American Society for Clinical Nutrition).

port of toxicity, more is toxic dose that can be particularly important as all have cancer and intervention trials are generally at high risk for level of toxicity.

Consumption of foods high in carotenoids in cancer prevention trials are reported to reduce cancer risk (4, 5) and reported by subjects to be associated with toxicity, carotenoder-

malacia, and carotenodermia. Carotenoid supplementation for cancer prevention in six treatment groups receiving 30 mg carotenoid groups and 12 mg/day β -carotene control group received controlled

carotenodermia was observed only in the 30 mg group. Figure 1 shows the changes in carotenoid levels over the course of the study. Carotenodermia was first observed typically was first observed. Carotenodermia was first observed and persisted at high levels of intake, peak carotenoid levels (mg/dl) in subjects with carotenodermia ranged from 115-150 mg/dl. Chemical abnormalities

are observed in evaluating toxicity of carotenoid supplements. The potential toxicity of carotenoid supplements (6, 7).

Carotenoid dietary changes can be used as a marker in addition, markers can be used to estimate the same nutritional status in other settings. Markers of carotenoid status and may thus be used to assess changes in various meta-

parameters to changes in dietary fat in premenopausal women, also conducted as part of the USDA-NCI collaboration, illustrates the use of markers of dietary compliance. In this instance, plasma cholesterol was evaluated as a potential marker of compliance for persons changing from a high- to low-fat diet (8).

Thirty-one premenopausal women participated in a controlled diet study in which they consumed a conventional high-fat diet (40% derived energy from fat) for four menstrual cycles and then switched to a low-fat diet (20% energy from fat) for an additional four menstrual cycles. One-half the subjects were maintained throughout the study at a ratio of polyunsaturated-to-saturated fatty acids ($P:S$ ratio) of 1.0, the other half at 0.3; body weight remained constant.

Results of total plasma cholesterol analyses from blood samples drawn at the end of the high- and low-fat controlled diets indicated that small but statistically insignificant reductions in plasma cholesterol occurred on the low-fat diets. The mean decline in the individual differences for the $P:S = 1.0$ group was 14.6 mg/dl, an 8% decrease from the average initial value of 182.1 mg/dl, while the mean decline for the $P:S = 0.3$ group was 11.6 mg/dl, a 6% decrease from the average initial value of 188.0 mg/dl. Figure 2 shows plots of the individual values by diet and $P:S$ group. Almost as many women had an increase in their plasma chole-

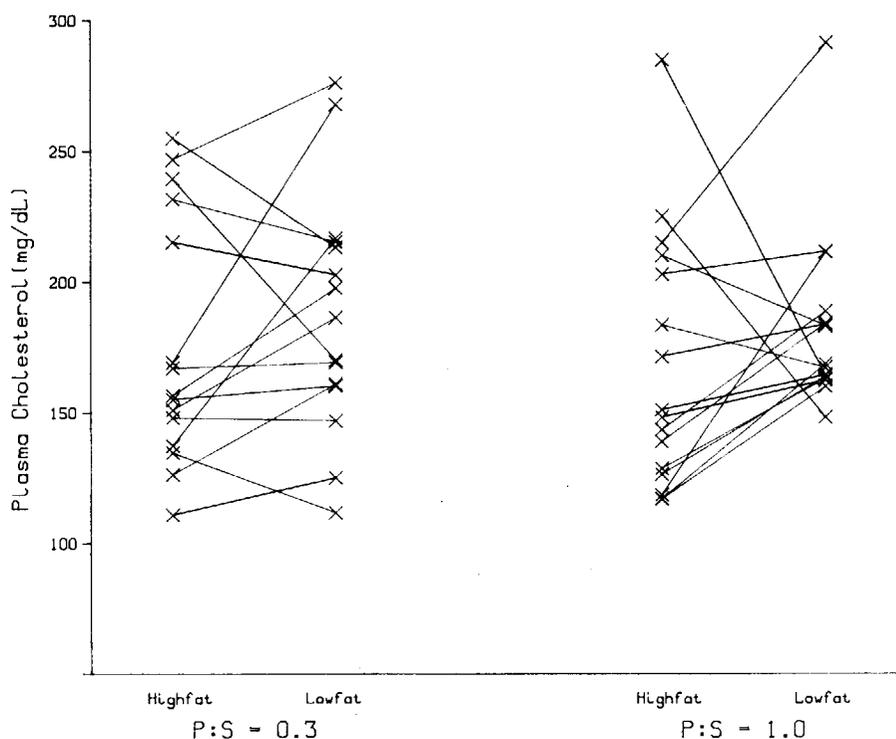


FIG. 2. Plasma cholesterol levels among individual premenopausal women when going from a high fat to a low fat diet.

terol on the low-fat diet as had a decrease. These findings indicate that plasma cholesterol is a poor marker of dietary fat in these women as individuals. It is possible, however, that in a large study of premenopausal women, plasma cholesterol may be a reasonable marker of group differences in dietary fat intake.

We have taken advantage of controlled diet studies in two circumstances to validate epidemiologic questionnaires. In the first instance, a quantitative food frequency questionnaire was administered to premenopausal women at the end of 14 days on a controlled high-fat diet, and again after 14 days on a controlled low-fat diet. Both times participants were asked to report intake over the previous 2 weeks, a time interval chosen because of the 14-day menu cycle used in the study. Questionnaire responses were compared with the known dietary intake to test the accuracy of subject recall. In the second instance, a series of questionnaires attempting to estimate physical activity were given to men participating in a controlled diet study, in which caloric intake was adjusted to maintain weight. Each of the physical activity questionnaires was then compared with known energy expenditure (i.e., energy intake).

Another instance in which such studies can be used to assess potential markers of dietary fat compliance is the collection and analysis of exfoliated cheek cells for fatty acids (9, 10).

MECHANISMS

Studies in which diet is manipulated and subjects are monitored for changes in selected biochemical parameters can provide valuable clues regarding possible mechanisms of action of diets or nutrients suspected to be carcinogenic. The dietary fat study in premenopausal women described previously was also used to evaluate the relation of changes in dietary fat to fecal mutagen levels, and illustrates this point (11).

Correlation studies suggest that fecal mutagenicity is increased in groups eating high-fat diets, the same groups who are often found to have high colorectal cancer incidence and mortality. The relation of fecapentaenes, the best characterized class of fecal mutagens, and dietary fat was previously unstudied in individuals. As part of the study, pooled 3-day stool samples were collected from women on each controlled diet during the periovulatory time of each woman's cycle in the second and fourth menstrual cycle. Stool samples from this period of the cycle were also collected during the free-living periods immediately before and 3-4 months following the controlled diet periods. Samples from all six collections were analyzed for fecapentaene excretion using high-performance liquid chromatography.

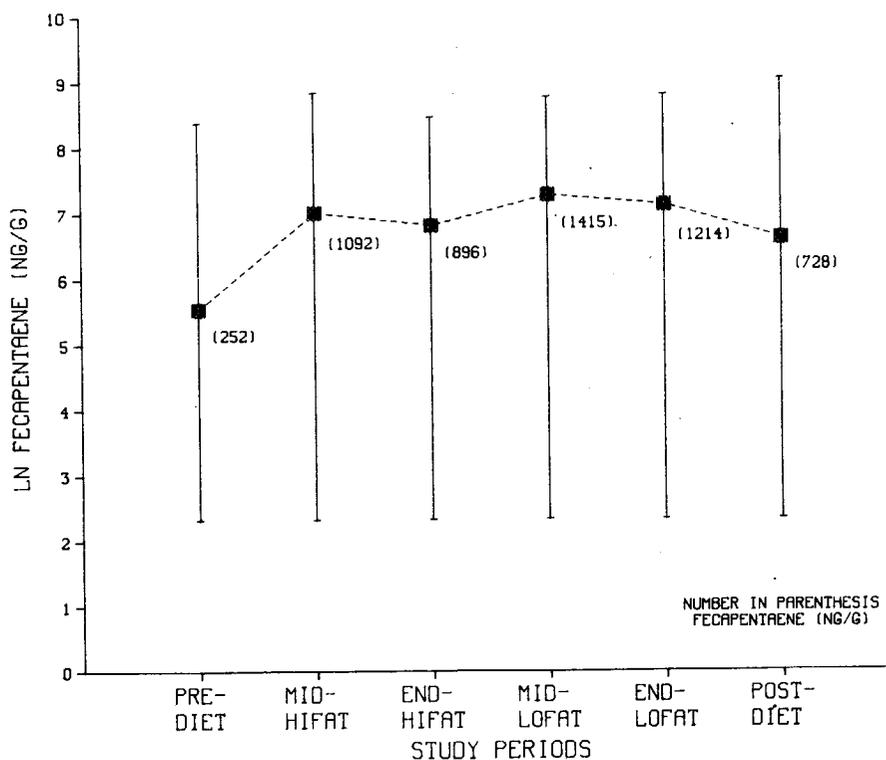


FIG. 3. Levels of fecapentaene in nanograms per gram dry stool by study period among premenopausal women (ln fecapentaene—median, 10th and 90th percentiles of distribution).

Median fecapentaene levels for each of the six study periods for the two P:S groups combined are shown in Fig. 3. No significant differences in levels were observed across the four measurements made during the controlled diets. A significant increase in fecapentaene was seen when going from the free-living prediet period to the controlled diets, and a nonsignificant decrease was seen when returning to free-living following the controlled diet periods. The study findings suggest that dietary fat does not explain fecapentaene levels, but that factors apart from fat may influence the fecal concentrations of these potent mutagens.

Other examples of the use of clinical metabolic studies in the evaluation of potential disease mechanisms in cancer research include the studies of Goldin *et al.* (12) and Schultz *et al.* (13) in which hormones levels thought to influence cancer risk were evaluated in relation to diet.

INTERMEDIATE ENDPOINTS

The fifth area in which clinical metabolic studies can be useful in cancer research is in the identification and evaluation of intermediate endpoints. An intermediate endpoint is defined as a marker of biologic damage or morphologic change and is distinguished, to the extent possible, from an exposure marker by being closer to disease in the overall exposure-disease relation. It might also be called a predisease or premalignant marker.

An example of a study with an intermediate endpoint is provided by the work of Lipkin and Newmark, who investigated the effect of calcium supplementation on cell proliferation in the mucosa of the colon (14). They evaluated the effect of 1.25 g of calcium as calcium carbonate taken for 2-3 months on the frequency and distribution of proliferating epithelial cells lining colonic crypts, as assessed by tritiated thymidine labeling. Their study group included 10 asymptomatic members of families having increased frequencies of colonic cancer without polyposis.

Results of the labeling studies showed a decrease in proliferative activity in all five colonic crypt compartments following calcium supplementation (Fig. 4). Overall, labeling decreased from 17 to 10% following treatment. Although this was a small study without a concurrent comparison group or strict dietary control, the results strongly suggest the need for further evaluation of the possible beneficial role of calcium in preventing colon cancer.

As additional illustrations, Stich *et al.* (15) investigated micronucleation of buccal mucosal cells and Gouveia *et al.* (16) studied bronchial metaplasia as intermediate endpoints in cancer studies.

STRENGTHS AND LIMITATIONS OF CLINICAL METABOLIC STUDIES

The major strength of clinical metabolic studies of diet and cancer is the level of detail and control that can be achieved in such studies. Exposure can be assessed thoroughly and, in the case of interventions, carefully controlled. Endpoints, typically biochemical or morphologic changes, can also be measured in an optimal manner. A variety of different biologic samples can often be obtained from the same individual, and measurements can be repeated over time.

The greatest weakness in metabolic studies from the point of view of cancer

periods for the two P:S differences in levels were controlled diets. A significant difference was seen when re-evaluated. The study findings suggest that factors apart from potent mutagens are important in the evaluation of the studies of Goldin *et al.* thought to influence

is useful in cancer research endpoints. An intermediate or morphologic exposure marker by definition. It might also be

provided by the work on calcium supplementation evaluated the effect of calcium on the frequency and number of crypts, as assessed by the number of asymptomatic members without polyposis. The proliferative activity in all crypts after supplementation (Fig. 4). Although this was not a strict dietary control, the possible beneficial

micronucleation of buccal metaplasia as inter-

METABOLIC STUDIES

and cancer is the level of exposure. Exposure can be fully controlled. Endpoints can also be measured in an animal often be obtained over time. The point of view of cancer

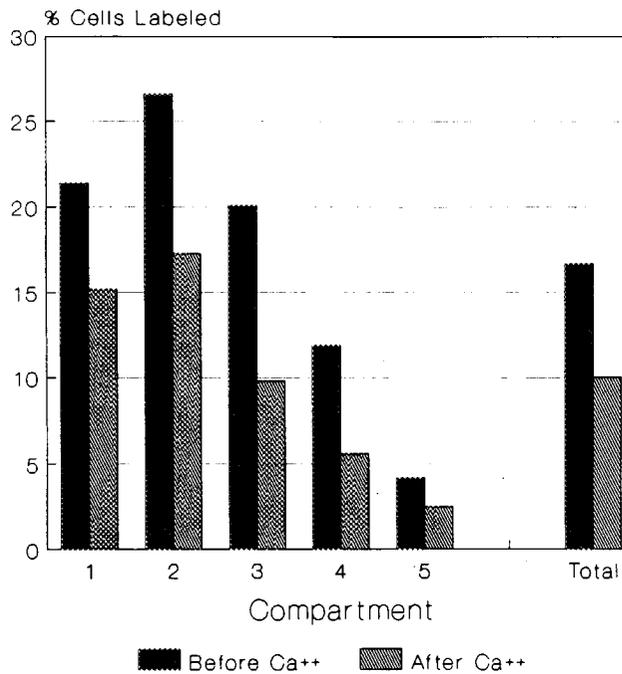


FIG. 4. Labeling indices for crypt compartments in colon biopsies before and after calcium supplementation. 1 = basal; 5 = surface. (Adapted from Lipkin and Newmark [14].)

research is that cancer is never the endpoint. These studies will be informative regarding cancer only to the extent to which our measurements relate to the exposure-disease pathway. The closer the study endpoint is to cancer, the more informative the study will be. Other limitations relate to the generally small size of such studies, their expense, and the need for special facilities.

SUMMARY

Information from clinical metabolic studies can be a valuable part of the broad approach to understanding disease. There are at least five areas in which clinical metabolic studies can be of use in cancer research: kinetics, toxicity, markers, mechanisms, and intermediate endpoints. Metabolic studies of this type are useful both to cancer etiology and prevention. However, the successful development of clinical metabolic studies will require extensive interdisciplinary collaboration between nutritionists, epidemiologists, clinicians, and laboratory scientists.

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