

*Hypothesis/Commentary***Diet and Cancer: The Disconnect Between Epidemiology and Randomized Clinical Trials**Frank L. Meyskens, Jr.¹ and Eva Szabo²¹Departments of Medicine and Biological Chemistry, Chao Family Comprehensive Cancer Center, University of California, Irvine, Orange, California; and ²Division of Cancer Prevention, National Cancer Institute, NIH, Bethesda, Maryland**Abstract**

Dietary epidemiology has been highly successful in identifying the responsible agent in many diseases, including scurvy, pellagra, blindness, and spinal bifida. Case-control, cohort, and ecologic observational studies have consistently associated increased consumption of fruits and vegetables with a decreased risk for a wide variety of epithelial cancers and, in many cases, specific dietary components seem to decrease the risk for a wide array of epithelial cancers. Over time, there has been enthusiasm for one or another compounds, such as β -carotene in the past and folate currently. Despite the success of translating similar epidemiologic observations to clinical benefit in other areas of medicine via the crucible of the randomized clinical trial, this strategy has not been nearly as successful for cancer. We propose that the inability of nutritional epidemiology to identify effective chemopreventive strategies is not just a problem of quantitation, but rather that the discipline is usually qualitatively incapable of identifying a dietary compound(s) that will be efficacious. One needs to consider the following basic questions in trying to understand why nutritional epidemiology has not been translated into progress in cancer prevention: Why do fruits and vegetable show a consistent protective effect against many epithelial cancers in epidemiologic studies? Once a specific dietary compound is identified as protective in observational studies, why do most subsequent observational studies confirm the effect? Why are dietary epidemiology observations frequently not confirmed by the randomized clinical trial? We call the identified problems "fishing with only one bait" and the "four-

legged stool problem." The considerations identified in this analysis offer a number of possible solutions to puzzling findings: (a) Fruits and vegetables consistently show a protective effect against cancer in observational studies because they represent the entire "biological action package." (b) Dietary compounds show a protective effect in observational studies, but not in clinical trials, because this is an inevitable consequence of one compound being falsely identified as the active agent in a system in which multiple agents or multiple interacting regulatory molecules underlie the biological effect. The consequences are serious for trying to use epidemiology to identify effective nutritional compounds. The major conclusion has to be as follows: Supplementation with single dietary compounds is rarely going to be as effective as epidemiologic studies suggest; it is the biological action package that determines efficacy. Options for how we should move forward will be discussed. Dietary observational epidemiology is complex and involves many biases and confounders. We need to be more critical in the design of large randomized trials based on observational epidemiology or analysis. Rules of evidence are frequently ignored or misunderstood although the limitations of observational epidemiology are analogous to the problems associated with discovery-based research and biomarker identification. We need to be much more self-critical in the important and critical assessment of dietary compounds and their role in cancer prevention given the very high appeal for this approach both within the lay and scientific communities. (Cancer Epidemiol Biomarkers Prev 2005;14(6):1366-9)

We have recently discussed the general topic of the limited success of chemoprevention trials in human cancer in terms of addressing two broadly posed questions (1):

- Why have we been relatively unsuccessful in translating promising epidemiologic and experimental findings to clinical benefit?

- How should we move the field of chemopreventive agent development forward in a manner that will be more productive?

Before one can fully answer the second question, it is important to consider the first one and to critically review previous attempts to identify clinically important chemopreventive strategies. Therefore, this commentary will address the problems associated with translating epidemiologic findings to clinical interventions and the associated question of whether the results of epidemiologic observations alone are ever enough to embark on a phase III trial.

An extremely informative analysis by Ioannidis et al. (2) compared the evidence for treatment effects in nonrandomized and randomized trials across a wide spectrum of medical conditions. Although the authors documented that the correlation between nonrandomized and randomized trials was fairly high ($r = 0.75$), the nonrandomized studies frequently overpredicted the magnitude of the effect by as

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Requests for reprints: Frank L. Meyskens, Jr., Hematology/Oncology, University of California, Irvine, Cancer Center, Building 44, Route 81, 101 City Drive Cancer Center, Orange, CA 92668. Phone: 714-456-6310; Fax: 714-456-5039. E-mail: flmeyske@uci.edu

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<u>Observational Trial</u>	<u>Intervention</u>	<u>Clinical Trial</u>
RR=0.7		

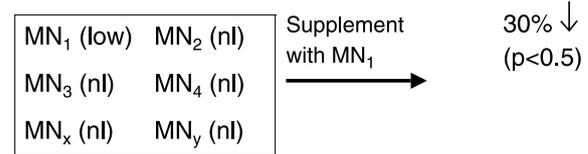
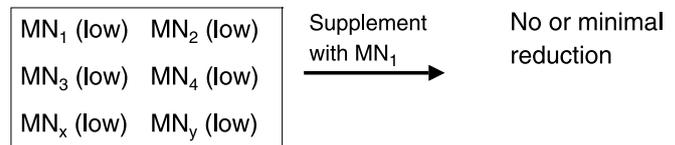
A Rare situation**B** Common situation

Figure 1. Observing the “black box” of dietary cancer epidemiology. **A.** Epidemiologic observations may correctly identify one micronutrient (MN_1) as causative when all the other known influencing micronutrients (MN_{2-4}) and unidentified micronutrients (MN_x, MN_y) that are not assessed are normal. This situation is likely to be rare. **B.** Epidemiologic observations usually identify one micronutrient as “causative” when, in fact, many others are involved in producing the event, and their levels or concentrations are abnormal. This situation is probably common. Supplementation in a clinical trial in the situation depicted in (A) may result in a beneficial effort, but is unlikely to occur for the situation in (B).

much as 50% to 100%. Because sample sizes for clinical trials are generally based on the relative risks (or the equivalent) shown in observational studies, there is a high likelihood that randomized trials are consistently underpowered, thereby missing a small, but real effect. Other factors also likely contribute to this divergence, including shorter exposure to interventions during randomized trials compared with exposure assessments in nonrandomized trials, incomplete compliance with interventional regimens, and drop-in and drop-out problems associated with clinical trials. Nevertheless, the question that arises is whether the issue of overprediction is more problematic for nutritional epidemiology and diseases having complex etiologies, such as cancer, in contrast to diseases having simple etiologies, and why this should be so. We propose that the inability of nutritional epidemiology to identify effective chemopreventive strategies is not just a problem of quantitation, but rather that the discipline is usually qualitatively incapable of identifying a dietary compound(s) that will be efficacious. In this commentary, we set forth the basis for this conclusion and discuss potential strategies to provide more useful data and, hopefully, to better define the questions that need to be considered before the next generation of cancer prevention trials based on epidemiologic evidence. Because methodologic issues and limitations of dietary cancer epidemiology assessment tools have been reviewed multiple times previously (3), these will not be discussed further; we will instead focus on theoretical limitations of the ability of epidemiologic investigation to identify efficacious dietary chemopreventives.

Epidemiologic observation has been highly successful in identifying the responsible agent in many diseases caused by specific nutritional deficiencies, such as scurvy, pellagra, blindness, and spina bifida. Similarly, multiple, although not all, case-control, cohort, and ecologic observational studies have associated increased consumption of fruits and vegetables with a decreased risk for a wide variety of epithelial cancers and, in many cases, specific dietary components have seemed to decrease the risk for a wide array of epithelial cancers (4-6). Over time, there has been enthusiasm for one or another compound, such as β -carotene in the past (7) and folate currently (8, 9). However, despite the success of translating similar epidemiologic observations to clinical benefit in other areas of medicine via randomized clinical trials, this strategy has not been nearly as successful for cancer (10, 11). Although calcium seems to reduce the number of colonic polyps in prone individuals (12, 13), many more dietary components have produced either no benefit or, as in the case of β -carotene in smokers, an adverse effect (14, 15). Why should this be the case? We propose that nutritional epidemiology has not been

translated into progress in cancer prevention because of inability to identify all relevant dietary components that act coordinately to produce any given result and because of inability to identify the other relevant nonnutritional factors that interact with dietary components to achieve any given outcome.

It is important to recognize that micronutrients or any other dietary components do not act in isolation, but as part of a package. Let us first consider the results of micronutrient replacement for deficiency within the context of differing levels of associated micronutrients (Fig. 1). An observational trial detects and associates the presence or level of one micronutrient (MN_1) with a cancer. As shown in Fig. 1A, only MN_1 is low whereas other micronutrients known to be associated with MN_1 ($MN_2, MN_3,$ and MN_4) are normal. Furthermore, additional micronutrients that impact on the same pathway as MN_1 but have not been identified (MN_x and MN_y) are also normal. When MN_1 is replaced by supplementation, as in a clinical trial, a comparable benefit to that predicted by the observational study is shown. The next and subsequent observational studies will continue to show a beneficial effect, as will a meta-analysis of the association of MN_1 with cancer. Not knowing the identity of all relevant micronutrients does not impact the success of the studies in this situation. However, this situation, in which only MN_1 deficiency occurs and leads to cancer in the absence of other micronutrient deficiencies, is probably rare.

The more likely real situation relating to micronutrients in individuals at risk for cancer is shown in Fig. 1B. MN_1 is low, but so are one or more other related micronutrients ($MN_2, MN_3,$ and MN_4), although the observer (the epidemiologist) is unaware of the deficiency of these other known dietary components. Furthermore, one or more unidentified micronutrients, MN_x and MN_y , which are not known to be involved in pathways impacted by MN_1 , may also be deficient. A corollary situation is that low MN_1 may simply track with low levels of another key micronutrient, and low MN_1 itself is of no importance whatsoever to carcinogenesis. In these situations, supplementation with MN_1 is likely to be futile, either because replacing one micronutrient in isolation is insufficient or because the wrong micronutrient is being replaced. “Unideficiency” diseases, such as scurvy, pellagra, and rickets, are easily correctable when the one micronutrient is replaced. Cancer, on the other hand, is highly unlikely to be a unideficiency disease and thus unlikely to be easily prevented from progressing by correction of a single deficiency. Whereas nutritional epidemiology can identify the deficient MN_1 , it may be much more difficult to appreciate the deficiencies in the associated micronutrients $MN_2, MN_3,$ and MN_4 , and nearly impossible to identify the unknown elements MN_x and MN_y .

Perhaps sharpening of the measurement tools of nutritional epidemiology may allow better identification of the elements that compose Fig. 1B, although it is possible that the tools may never be accurate or specific enough to identify all relevant dietary components. This concept can be summarized by an analogy to the fisherman who uses only flies of interest to trout as bait and never discovers that bass, pike, or sturgeon also swim at the bottom of the lake. He will continue to catch many trout each time he throws out the flies meant to catch trout, but he will miss the diversity of the lake population. If one cannot determine the identity of critical nutritional components, appropriate replacement strategies cannot be tested. Thus, better and more accurate measures of dietary intake and, critically, of their biological effect in specific organ sites, are necessary for progress to occur. On the other hand, if we keep over fitting the data, we are only going to identify the same dietary components as previously. In this regard, larger cohorts are not going to give us new information because the biases are all in the same direction. Thus, these observations present a paradox in that it seems important to focus nutritional epidemiology tools to allow identification of specific important dietary components and yet what one might ultimately want is a very general way to identify multiple important components simultaneously. The immediate solution to this conundrum is unclear.

More accurate objective measurement (? biomarker) of dietary intake and its defining effects on carcinogenesis in a particular tissue should provide a better definition of the importance of any dietary compound in the human situation. Such information should enhance the likelihood that a single dietary supplement would result in a positive outcome in a clinical trial. Alternatively, multicomponent supplementation or food trials might be worth reconsideration because the reductionist approach has not been particularly successful. The positive results of two large trials in a cohort at risk for gastric/esophageal cancer suggest that a multicomponent approach should be more broadly explored (16, 17).

A more serious limitation of nutritional epidemiology, however, is the failure to measure (and possibly appreciate) what we call the "biological action package." Almost all dietary compounds have a diversity of biological/biochemical effects. Equally important and frequently ignored is that the action of a dietary compound is strongly affected by the biochemical milieu in which it resides, a feature significantly influenced by organ or tissue site. Therefore, the full benefit of dietary component replacement may not be realized if other components of an effector pathway (or pathways) are abnormal.

Factors involved in optimizing the biological effects of a dietary compound include both "upstream" (e.g., absorption,

metabolism) and "downstream" (e.g., effector molecules) influences, as well as interactive and parallel pathways. This concept is exemplified by analogy to a four-legged stool, where all four legs of the stool (the multiple components of the biological action package) are involved in keeping the stool erect and functional, and propping up only one leg does not fix the problem if one or more of the other legs remains deficient. As an example, consider any dietary compound that is involved in redox regulation. One cannot get a true understanding of the action of a dietary compound without examining other major components of redox regulation, such as glutathione, thioredoxin, superoxide dismutase, and many others (18). The biochemical status and composition of key biochemical components in an organ may be even more important in determining the effect of a specific micronutrient than are other micronutrients.

To return to the hypothetical situation involving micronutrient MN_1 , consider Fig. 2A, where MN_1 is low and the biochemical components that interact with MN_1 are normal. Whereas this situation is likely to be rare, supplementation with MN_1 may actually result in reduction of events in a clinical trial, particularly if it produces a strong biological effect. Much more likely is the situation in Fig. 2B, in which MN_1 may be low and several other key components of the biological action package are also low or out of balance, although their status is not appreciated or measured. Once again, components include those that are important (such as the redox regulators glutathione, thioredoxin, etc.) as well as factors not yet known to be important (labeled *F1* and *F2* in the figure). Supplementation with MN_1 in this case may allow a small nonsignificant reduction of risk to be shown in the clinical trial, or, more likely, no effect will be evident.

Identifying the other key regulatory components of the biological action package by a variety of biochemical or molecular analyses may allow more precise epidemiologic estimation of risk, but the more difficult assessment that remains is the determination of the changes that the disease process itself produces. Powerful new technologies such as microarray analysis or proteomics may help to advance the science of basic nutrition such that nutritional epidemiology can appropriately focus on the entire biological action package. As is more frequently the case in science today, cross-disciplinary efforts involving epidemiologic studies, animal model systems, and molecular analyses focusing on *in vivo* as well as *in vitro* models are more likely to move the field forward.

The considerations discussed in this commentary offer a number of possible solutions to puzzling findings. We propose that: (a) Fruits and vegetables frequently show a protective

Observational Trial

RR=0.7

A Rare situation

MN_1 (low)	GSH (nl)
TX (nl)	SOD (nl)
F1 (nl)	F2 (nl)

Supplement
with MN_1 →

30% ↓
($p < 0.5$)

B Common situation

MN_1 (low)	GSH (low)
TX (low)	SOD (low)
F1 (low)	F2 (low)

Supplement
with MN_1 →

No or minimal
reduction

Figure 2. The biological action package involves more than dietary compounds. **A.** MN_1 is assessed and found to be low, whereas the other major influencing biochemical parameters are normal. In this example, the redox pathway is highlighted with identification of known components glutathione (GSH), thioredoxin (TX), and superoxide dismutase (SOD). The unknown components of redox regulation are identified as F1 and F2. **B.** MN_1 is assessed and found to be low, whereas the other major influencing biochemical parameters are also abnormal. Epidemiology usually sees only one component of the biological action package. In the situation in (A), which is likely to be rare, supplementation with MN_1 results in a beneficial effect in a clinical trial. However, in the situation in (B), which is likely to be more common, supplementation with MN_1 shows no or minimal effect.

effect against cancer in observational studies because they represent the entire biological action package. (b) Dietary compounds show a protective effect in observational studies, but not in clinical trials, because this is an inevitable consequence of one compound being falsely identified as the active agent in a system in which multiple agents (Fig. 1B) or multiple interacting regulatory molecules (Fig. 2B) are responsible for the biological effect.

Other concerns in the translation of epidemiologic observations to clinical trials also need to be mentioned. Clinical interventional trials require the use of well-defined compounds at specified doses and for specified durations of time. Epidemiologic data rarely defines these parameters exactly, particularly the duration of replacement therapy and the timing of replacement during the lengthy process of carcinogenesis. Furthermore, pharmacologic replacement frequently involves using a specific compound or compounds with subtle differences from the physiologic entity and at doses that do not necessarily reflect physiologic levels. Hence, pharmacologic replacement may differ significantly from dietary supplementation. The idea to replace specific dietary components may be correct, but the formulation in a clinical trial may be faulty and lead to failure. The adverse results seen in the clinical trials of β -carotene in smoking individuals may well represent such a case, because the doses used were three to four times greater than the upper limits of the dietary intake and the levels of serum β -carotene obtained were nonphysiologic (14, 15).

The concepts discussed in this article have consequences for trying to use epidemiology to identify effective nutritional compounds. Our major conclusion has to be that supplementation with single dietary compounds is rarely going to be as effective as epidemiologic studies suggest because it is the biological action package that determines efficacy.

Having identified some of the caveats associated with the translation of epidemiologic observations to clinical trials, the most important question is how should we move forward? Options include the following: (a) Increase the sample size of single-agent trials; (b) Stop performing trials with single agents and do only studies with combinations of compounds. If so, how many and which agents should be studied?, or (c) Reconsider doing food supplementation or broad dietary change clinical or population based studies. However, how does one standardize food sources grown in different areas or in different soils, and how does one change behavioral and cultural habits such as diet? Moving to multiagent, food supplementation, or lifestyle change trials brings an entirely new series of complexities to trial design and execution, although that should not serve as an excuse for not performing the appropriate studies. Recognizing the importance of dietary patterns in chronic disease prevention and the limitations of current knowledge, recommendations for a broadly based research agenda in this area have recently been proposed (19).

So, what should one do? First, recognition of the issues outlined in this commentary should lead to a healthy debate and better definition of the problems associated with translation of epidemiologic data to clinical intervention. A major issue to consider is whether the scientific community is willing to take a more public health approach in addition to rethinking the reductionist medical approach in the matter of diet and cancer. In other words, do we really need to know which components of food are the active agents if changes in diet will result in reduction of cancer incidence or risk in the population at large? Second, although food and agricultural science is far advanced, the basic science of nutrition and its role in carcinogenesis has lagged behind and needs a similar level of understanding. The development of consequential markers of dietary intake/effect on carcinogenesis in the relevant organ is

critical. The rationale for single-agent supplementation needs to be carefully defined before proceeding to large trials. Careful consideration should be given to preferentially performing combination or food trials.

These issues need to be discussed and debated before we launch into the next generation of cancer prevention studies based on epidemiologic observations. Dietary observational epidemiology is complex and involves many biases and confounders. We need to be more critical in the design of large randomized trials based on observational epidemiology or analysis. We need to be much more self-critical in the important and critical assessment of dietary compounds and their role in cancer prevention given the very high appeal for this approach both within the lay and scientific communities (20).

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