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Nutritional epidemiology: forest, trees and leaves

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Abstract
Ioannidis has stated that the field of nutritional epidemiology has generated confusion and numerous implausible findings and is in need of radical reform. One of the reforms he proposes is to conduct analyses that take into account the "totality for all nutritional factors measured". This approach is based on isolating and reducing diet into numerous independent variables with little regard to prior knowledge or the interrelations among dietary components, and relying on a "discovery" approach. This method, akin to genomewide association studies (GWAS), would involve very large sample sizes, small associations, no prior knowledge, and multiple testing considerations. This method is contrary to the more traditional hypothesis generating and testing approach built on all types of evidence. This commentary will contrast how suitable these two approaches are to study diet and disease.

Keyword Nutrition · Epidemiology · Cardiovascular diseases · Diet

Introduction

Ioannidis has criticized the current status of nutritional epidemiology [1–3], and has stated that the field needs "radical reform." This conclusion is based on the complexity of diet, the inadequate ways we have to assess diet, and the presumably intractable problem of confounding. In his assessment, the present practice of nutritional epidemiology has led to confusion and numerous implausible findings. One of the main reforms he proposes is to conduct more randomized trials (RCTs), which is discussed elsewhere [4]. Another reform is to make cohort data available for reanalysis by independent investigators and conduct analyses that take into account the "totality for all nutritional factors measured", to limit false positives from multiple testing, akin to genome-wide association studies (GWAS) [5]. This commentary will contrast two approaches to study diet and disease: (1) isolating and reducing diet into numerous independent variables with little regard to prior knowledge and using a "discovery" approach (GWAS-like), or (2) a hypothesis generating and testing approach built on layers of evidence.

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Diet is extremely complex to assess and study but manageable in the context of subject-matter focused hypothesis testing

Ioannidis describes diet as composed of thousands of chemicals in millions of possible daily combinations from 250,000 different foods, superimposed on complex interactions with age, genetics, and other factors. He then states "… limited self-reported nutrition data ascertained with a handful of questions and self-reported items fail to acknowledge or accurately measure a system that matches or exceeds the genome in complexity". Is the analogy of diet and the genome a realistic one? I believe diet and the genome have major differences that affect how they are best studied in regards to health. There are up to 10 million common variants in the genome (> 5% allele frequency) that everyone is exposed to. The genome was shaped largely before the modern era of major non-communicable diseases, which had been rare regardless of genomic variability. We have little a priori expectation that any given variant is associated with a specific disease. A GWAS "discovery" approach makes sense in this scenario. This approach involves very large sample sizes, small associations, no prior knowledge, and adjustment for multiple testing.

Diet has a different connection to disease. Among diet, among the 250,000 potential foods that exist globally, the exposure is zero or close to zero for 99.99%. The vast

majority of these are in the plant kingdom, and many people hardly eat any fruits and vegetables. What would be the point of adjusting *p* values for the millions of compound in the rare foods that hardly anybody eats when examining health effects for a specific hypothesis? In stark contrast to the genome, we are primarily interested in the massive changes in diet, as well as physical activity, which have occurred in the twentieth century. For public health questions focused on behavior-related exposures, especially those that have changed dramatically in a short time, a hypothesis based approach makes more sense. Tobacco is a better model for diet than the genome. The sole fact that many people smoke tobacco makes it relevant to study. The potential effects of thousands of other leaves that can be burned and inhaled are irrelevant to public health (except for a few others, such as marijuana).

While more multidimensional than smoking, diet may be comparable to smoking in that we have generated essentially novel, potentially deleterious dietary exposures, unseen in the evolutionary history that shaped our genome. Through changes in the food supply, we have altered diet profoundly. For example, we have inverted the potassium/sodium (K/Na) ratio from around 16 to often below 1 [6, 7]; altered the polyunsaturated/saturated (P/S) ratio from about 1.4 to well below 1 [6, 7]; introduced partially hydrogenated trans-fat [8], a fat almost ideally designed to induce atherosclerosis; isolated and refined starch, sugar and fat, allowing fiber and nutrient-depleted calories to be massively consumed. Moreover, sedentary behavior has skyrocketed, amplifying some of the adverse health effects of the dietary changes through insulin resistance.

Not surprisingly, according to Global Burden of Disease Study 2016, in 113 countries in 2016, the leading risk factor in terms of attributable disability-adjusted life-years (DALYs) was a metabolic risk factor [9]. Table 1 fall lists a sampling of some potential questions related to substantial changes in the diet that may contribute to health. Answering just these questions would be of immense public health importance and greatly inform on dietary guidelines.

The current methodologies, particularly food frequency questionnaires, can address major dietary questions

If one considers addressing such public health dietary questions as an important goal, what is the appropriate methodology? Short-term interventions demonstrating a dietary effect on important disease intermediates inform on what dietary factors may be important, but do not examine directly long-term intakes on hard endpoints in large populations. Food frequency questionnaires (FFQs) have emerged as the most feasible approach for this endeavor, complemented by nutritional related biomarkers, and now by some -omics (e.g. metabolomics). The FFQ is designed to be informative regarding major nutrients, food groups and dietary patterns. Typical FFQs may contain 100–200 items, which cover the vast majority of foods consumed in its targeted population. The granularity of the FFQ is affected by combining foods (e.g., different types of apples), but this may not make a large difference on informing on recommendations (e.g., to eat more apples). The extensive evidence that dietary assessments are reasonably accurate to capture this level of detail (e.g., broad dietary patterns, major food groups, major nutrients) is reviewed elsewhere [10]. Well-designed FFQs provide information on long-term intakes with the approximate precision level as a one-week diet record [11].

While potentially useful, FFQs incur significant measurement error. Thus, a key question regarding their utility is what magnitude of association are we trying to detect? Again, we can use the example of tobacco, where a single or a few questions on cigarette use are adequate to easily detect strong deleterious effects of smoking. We don't require a complete understanding of the estimated 7000 chemicals including carcinogens from cigarette smoke before initiating public health actions regarding smoking. The major dietary changes that have affected health have occurred relatively recently, are widespread, and have strong effects, giving nutritional epidemiologists

Table 1 Some representative questions that are important to address regarding nutrition and health

Is replacing saturated fat with polyunsaturated fat beneficial?
Is carbohydrate quality (whole grain, high fiber vs. highly refined, low fiber carbohydrates) important?
Do dietary patterns that raise the potassium/sodium ratio lower blood pressure and CVD?
Is reducing intake of red and processed meat in lieu of other protein sources beneficial?
Do certain dietary patterns, in combination with physical activity level, influence weight gain?
Is intake of trans fatty acids deleterious for CVD risk?
Does intake of omega-3 fatty acids (from fish) matter for CVD risk?
Is high intake of sugar (especially sugar sweetened beverages) deleterious for metabolic diseases?
Do dietary factors that influence glycemia, insulinemia, and inflammation influence diabetes risk?
Do dietary factors that influence hyperinsulinemia influence risk of obesity-related cancers?

large targets, identifiable within the precision of the FFQ. FFQs may not always be able to address all hypotheses; biomarker and -omics data may help in some cases. Yet, these approaches should be considered complementary because they typically do not inform directly on dietary intake, which is of most interest for recommendations, and are influenced by absorption, metabolism, homeostatic mechanisms, other dietary factors, among others.

Confounding is important but manageable if diet-disease associations are approached from a hypothesis-testing strategy

Ioannidis states "… given the complicated associations of eating behaviors and patterns with many time-varying social and behavioral factors that also affect health no currently available cohort includes sufficient information to address confounding in nutritional associations." Confounding is an important issue in any observational study. For nutrition, it is useful to think of confounding at two levels. The first consideration is whether any observed diet association is independent of non-dietary lifestyle factors, such as smoking. We can deal with confounding, as for other lifestyle factors, through multivariable analysis. There is no reason to think that diet is inherently different from other behavioral-related factors generally accepted as causally associated with various outcomes. For example, smoking, alcohol, sexual behaviors, sun exposure, and physical activity are robustly associated with health outcomes; these certainly are linked to social and behavioral factors.

The second aspect of confounding is that among the dietary components itself, especially for highly correlated compounds in similar foods. For example, beta- and alpha-carotene are both high in carrots and common foods, making them highly correlated. However, focusing on a circumscribed number of important hypotheses (as in Table 1) is a useful and manageable approach for confounding. Ultimately, when "diet" is defined by a whole dietary pattern, the problem of confounding among individual dietary components is largely eliminated because we reduced the whole of diet into a single all-encompassing variable (e.g., Mediterranean diet). In this manner, rather than attempting to tease out the multitude of independent factors, as well as their interactions, synergies, etc., we embrace these interrelationships and consider the overall dietary pattern as the exposure. While in principle countless dietary patterns are plausible, we can use prior data and judgement to test a specific composite diet (e.g., Mediterranean diet) for practical guideline formation.

The main results yielded from nutritional epidemiology are coherent

Ioannidis asks "…can small intake differences of specific nutrients, foods, or diet patterns with similar calories causally, markedly, and almost ubiquitously affect survival?" To support his quote, he cites a meta-analysis that shows inverse associations with total mortality with increasing intake of whole grains, vegetables, fruits, nuts, and fish, and increased risk with higher intake of red meat and processed meat [12]. Do these results seem plausible? I suggest that they are for the following reason: 4 major health risk factors, blood pressure, lipid profile, blood glucose and insulin, are all strongly influenced by precisely the dietary factors mentioned by Ioannidis. A simplified model linking certain foods to these intermediates and major disease is presented in Fig. 1. There are considerable RCT data linking the dietary items to the intermediates, which are among the most robust predictors of major diseases. Although the RCTs linking diet to the intermediates are relatively short-term, there is no physiologic reason why these effects would disappear with long-term adherence. Nutritional epidemiology fills in the gap linking long-term adherence to diet to the hard endpoints.

For blood pressure, it is well established by physiologic studies and in meta-analyses of multiple RCTs that an increase in the dietary potassium/sodium (K/Na) ratio, will substantially decrease blood pressure, an extremely important factor in CVD and thus death [13–16]. Hence, diets that increase potassium intake (fruits, vegetables, whole grains, low-fat dairy, nuts) and lower sodium intake (avoidance of various highly processed foods enriched with salt) should lower blood pressure. Additional dietary factors, mostly in fruits and vegetables, other than the K/Na ratio, may also be important [17]. No surprise that in nutritional epidemiology studies, these dietary factors are associated with lower risk of CVD [12, 18, 19].

Regarding lipids, a Presidential Advisory for the American Heart Association concluded "Taking into consideration the totality of the scientific evidence, satisfying rigorous criteria for causality, we conclude strongly that lowering intake of saturated fat and replacing it with unsaturated fats, especially polyunsaturated fats, will lower the incidence of CVD" [20]. Independent meta-analyses support this conclusion [21]. One would anticipate that eating more foods that increase the polyunsaturated/saturated (P/S) fat ratio (nuts, vegetable oils) and fewer foods that decrease this ratio (fatty red meat, high fat dairy, many processed foods, especially with trans-fat) to be associated with reduced CVD incidence and mortality. No surprise from nutritional epidemiology [22–24]. In pooled data from 11 American and European cohort studies, replacing

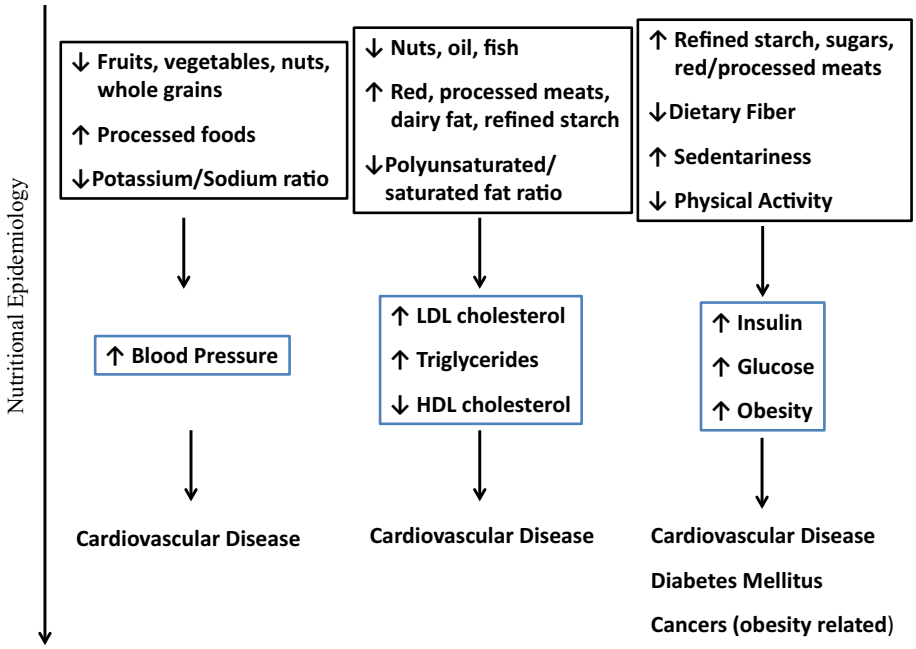

Fig. 1 Schematic summary of some of the major dietary factors and their mediators to influence risk of cardiovascular disease, diabetes mellitus and some cancers. This paradigm is built on shorter term studies linking the dietary factors to the intermediates, studies linking the intermediates to the diseases, and nutritional epidemiology associating the dietary factors to the diseases

saturated fat with polyunsaturated fat rather than monounsaturated fat or carbohydrates was associated with a lower risk of coronary heart disease over a wide range of intakes [22]. Although additional effects of dietary fats other than through plasma lipids are possible, the epidemiologic findings are largely in line with what is predicted by certain dietary factors of LDL cholesterol, HDL cholesterol and triglycerides. For example, refined carbohydrates are not an ideal replacement for saturated fat because of their deleterious effects on HDL cholesterol and triglycerides. RCTs with hard endpoints, while limited, are also consistent with the relative benefits of unsaturated fats and the insignificance of total fat as a percentage of energy [20, 25–27].

The glycemic and insulinemic arms are undoubtedly important regarding metabolic syndrome disorders including type 2 diabetes (DM). While diet, especially overeating in general, is obviously critical, isolating very specific factors is difficult, likely because there are several overlapping ones. Diets high in processed sugar, refined starch, and added (saturated, trans-) fats likely contribute, interacting with physical activity and sedentariness. Interestingly, this arm, while relevant for CVD, is particularly important for DM and some cancers, especially the obesity and physical activity related cancers [28]. Again, there is no surprise in the factors that show up in nutritional epidemiologic studies.

Diet, physical activity and body adiposity are inter-related

Ioannidis states "Unfounded beliefs that justify eating more food, provided "quality food" is consumed, confuse the public and detract from the agenda of preventing and treating obesity," implying that dietary recommendations encourage people to keep eating "low quality" food but to add more "high quality" food. The worry that recommending eating too many green, leafy vegetable and apples will contribute to obesity seems strange. In fact, a diet based on replacing low "quality food" with "high quality" food may be one of the most important ways of preventing obesity [29]. Generally, eating more of the healthful items typically means less of the "obesogenic" foods. The massive change in the availability and quality of foods is generally accepted as one of the key drivers of the global obesity rates [30–32]. Higher quality dietary patterns versus lower quality patterns will tend to have more satiating components (lower energy density, higher fiber, higher protein) and fewer that induce glycemic and insulinemic spikes (i.e., processed sugar, refined starch, and added saturated fats). The overall "package" of these work effectively to mitigate weight gain; we will leave it to the reductionist to obsess over the independent causality for each individual factor. The consistent pattern that whenever abundant foods high in refined sugar, processed starch, and added fats become available, obesity rates invariably skyrocket in the populations is compelling [33, 34], although not the type of evidence that fits neatly into evidence-based paradigms and pyramids.

The debate on the relative importance of physical inactivity and sedentary behavior is unproductive and reductionist and often one-sided depending on the perspective. Both diet and physical activity are important, and in fact, they are interactive [35]. For those training for marathons or triathlons, the energy expenditure is high enough that energy balance is generally achievable regardless of diet quality. Maintaining a very lean body will mitigate (though not completely) some of the aspects of poor diets, particularly insulinemic and glycemic effects. Unfortunately, a large segment of the population will fall into the range of (very) sedentary, where the effects of poor diet quality on some intermediate markers and ultimately health will be amplified. For example, obese people with underlying insulin resistance have more pronounced glycemic and insulinemic responses and inflammation due to poor diet, and appear to have amplified risk of related chronic diseases [28, 36–39]. Thus, poor dietary quality has obesity-independent effects on health, very likely contributes to obesity, and amplifies many of the adverse biologic effects of obesity (e.g. on metabolic hormones, inflammation).

Reductionist approaches can still be useful in some circumstances

It seems highly unlikely that a few nutrients taken at high doses in pills will incorporate the major health effects of dietary patterns. Nonetheless, some benefits of selected micronutrients are possible as it is unlikely that micronutrient intakes are optimal for all populations for all circumstances. Although the "failures" (e.g., beta-carotene, vitamin E) are typically emphasized and oversimplified, some successes are less appreciated. An important example is folic acid (folate) and neural tube defects [40] and stroke. A potential benefit of folate on risk of stroke was suggested in epidemiologic studies [41, 42]. In subsequent RCTs conducted in countries that mandate folic acid fortification of food, folic acid supplementation did not reduce stroke risk, but in countries without mandatory fortification, folic acid supplementation reduced the risk of stroke by about 15–25% [43]. In the US and Canada, a reduction in stroke mortality occurred after folic acid fortification in 1996–1997 [44]. Calcium supplementation is likely to lower risk of colorectal adenomas and cancer [45]. Although more work is required, initial RCTs suggest vitamin D may lower risk of respiratory infections [46], asthma [47], otitis media [48], and possibly cancer mortality [49, 50], and observational studies and Mendelian randomization data suggest a role on multiple sclerosis [51, 52].

Although our main focus should be on overall dietary patterns, the discovery of single nutrients effects on important disease endpoints should not be abandoned. Even modest effects are important because the intervention is feasible. Candidates will arise from various sources, including animal studies, epidemiologic studies, identifying unique compounds consumed in specific cultures, and exploratory nutrient wide approaches.

Conclusion

Ioannidis states "Proponents of the status quo of nutritional epidemiology point to occasional small trials with surrogate or metabolic outcomes (e.g., lipids, diabetes, composite end points) whose results agree with epidemiologic findings. However, these small trials often have selective reporting bias similar to that of nutritional epidemiology." This statement is not true. The described dietary effects on lipids (fat quality on LDL and HDL cholesterol), refined carbohydrates on triglycerides and HDL cholesterol, blood pressure (fruits, vegetables, K/Na ratio), and various dietary factors interacting with physical activity on glycemia and insulinemia are as established as any in biomedical science. The patterns summarized in Fig. 1 show remarkable coherence in the factors predicted to influence CVD, DM and obesity-related cancers—for example, factors related to blood pressure (fruits, vegetables) and lipids (dietary fat quality) are less important for cancer risk, for which factors associated with obesity and hyperinsulinemia predominate as risk factors.

The list of hypotheses/questions in Table 1 are the "low hanging fruit", courtesy of the modern diet and lifestyle, and feasible for nutritional epidemiology to address. These are likely to be major dietary factors related to health, but important questions remain. For example, we have learned much about fat and carbohydrate quality, but the optimal quantity and quality of protein is less clear. We believe fruits and vegetables overall are healthful likely because many contain common beneficial factors (e.g., potassium, magnesium, fiber, vitamins), but whether certain groups (e.g., green leafy vegetables, citrus fruit, cruciferous vegetables) have unique benefits is not completely settled. Some evidence suggests that fruits and vegetables broadly are associated with lower risk of CVD, possibly because they are all high in potassium and low in sodium, but possibly very specific types with unique phytochemicals may be protective for some cancers [16]. We are not finished with discovery, but it is critical to not lose sight of the forest, become confused by the trees, and go searching leaf by leaf.

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