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Bioavailability of micronutrients obtained from supplements and food: A survey and case study of the polyphenols

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Abstract

Nutritional status is an important determinant of quality of life, morbidity, and mortality. This review is a survey of one of the least appreciated and understood factors that contributes significantly to nutritional health: that of bioavailability. As the economic importance of nutritional supplements and foods carrying claims of health promotion continues to grow, physicians are increasingly accountable for critically evaluating the therapeutic and toxicologic impact of any recommended nutritional supplements and foods, and to do so, an understanding of bioavailability is essential. As we learn more about nutrition, and as it becomes increasingly clear that our fund of knowledge about nutrition is not what it should be, physicians, allied health practitioners, patients, and public health policy makers are obliged to better understand the basis for efficacy and of safety of nutritional supplements and foods. The concept of bioavailability is central to advancing our clinical acumen, particularly for the older adult population (>55 years of age), which according to the Centers for Disease Control and Prevention, typically take 16 scripted medications daily. In addition, over half of all adults consume one or more dietary supplements (http://www.cdc.gov/nchs/data/databriefs/db61.pdf). The World Health Organization data demonstrate that older adults form the single largest demographic group at disproportionate risk of inadequate diet and malnutrition (http://www.who.int/ageing/publications/global_health.pdf) followed by the pediatric population (http://data.unicef.org/nutrition/malnutrition.html). The challenge and the importance of understanding the determinants of bioavailability and the fundamentals of toxicology are demonstrated through the consideration of this construct in polyphenols. In support of this review, we scanned the literature using PubMed and Google Scholar. We selected peer-reviewed studies and review papers using the following search terms: bioavailability, nutritional supplements, food matrix, polyphenols, flavonoids, toxicology, microbiome, dietary intake, and metabolism.

Keywords

Bioavailability, nutritional supplements, food matrix, polyphenols, flavonoids, toxicology, microbiome, dietary intake, metabolism

Introduction

According to published estimates, the global nutrition and supplement market stood at US$96 billion as of 2012. A year later, it was approximately US$104 billion globally.[1] This near spectacular growth of the nutritional supplement market underscores the primacy of the associated public health questions and issues. One of the central issues is the bioavailability of micronutrients from various sources. In contrast to the world of pharma, that is, drugs, there is a paucity of research on the bioavailability of micronutrients. This research void is especially salient for any meaningful

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understanding of bioavailability of critical micronutrients contained in nutritional supplements as compared with those in the food matrix.

The present work reviews principles of pharmacokinetics as they relate to the impact of dietary supplement and food matrices on micronutrient bioavailability and address the distinction between nutrient content and biological relevance. We examine a single class of compounds, the polyphenols. Their possible role in the management of diverse pathologies such as cancer and cardiovascular diseases is emerging and our understanding of the health effects of polyphenols clearly depends on their bioavailability and subsequent utilization in target tissues.

Reviewing the basics: Supplement versus food

The US Food, Drug, and Cosmetic Act [FD&C Act 201(ff)(1)] defines a dietary supplement as a product "intended to supplement the diet" that contains one or more of the following: a vitamin, mineral, herb or botanical, or amino acid, or "a dietary substance for use by man to supplement the diet by increasing the total dietary intake" or a "concentrate, metabolite, constituent, extract, or combination of above."

In contrast, food is far more broadly defined [FD&C 201(f)] as "articles used for food or drink for man or other animals, chewing gum, and articles used for components of any other such article."

Thus, supplements are specific adjuncts to food. It is important to note also that what is implied and intrinsic to the spirit of the definitions is that nutrients and foods are dietary components that are consumed orally and require supralingual mixing, mastication, and esophageal transit into the stomach and small bowel for further digestion or absorption. Each of the two broad classes of dietary components identified here (supplements and foods) contain micronutrients that play critically important roles in human health and disease.

Micronutrients are only required in trace quantities but participate in orchestration of a vast array of biological processes that drive normal growth, development, and function. They include but are not limited to vitamins, phytochemicals, and minerals such as iron, cobalt, chromium, copper, iodine, selenium, zinc, and chromium. Their bioavailability is conventionally defined as the fraction of a given dose of unchanged nutrient that reaches the systemic circulation. For dietary supplements, herbs, and other nutrients in which the route of administration is putatively oral, semantics suggest that bioavailability designates the quantity or fraction of the ingested dose that is absorbed.[2]

Bioavailability for dietary supplements can be further examined in two parts, as the proportion of the administered substance capable of being absorbed and that available for cellular uptake, use, or storage.[3]

Host factors in bioavailability

Human host physiology and metabolism is not only exquisitely complex but highly variable within a subject temporally and between subjects as a function of an array of variables:

- fed or fasted state of the individual;
- dietary load, source, and food matrix;
- general health and state of gastrointestinal tract (e.g. rate of gastric emptying, motility status, time of day, and lifestyle factors);
- acute or chronic illness of the individual (e.g. hepatic insufficiency and impaired renal function);
- pregnancy;
- status, if hospitalized (e.g. critical care unit);
- Postoperative status (e.g. active recovery from surgical intervention and wound healing stage);
- athletic status (e.g. ongoing athletic training or weekend athlete);
- nutritive status (e.g. malnutrition or nutrient insufficiency);
- age extremes (e.g. in general, drugs are metabolized more slowly in fetal, neonatal, and geriatric populations); and
- genetic makeup of the individual.

Food factors in bioavailability

The physicochemical dietary scaffolding has a profound impact on the way in which the host processes micronutrients and also in the chemistry of digestion, absorption, and distribution.

- *Presence and content of other nutrients*: For example, high levels of Zn decrease Fe and Cu absorption; vitamin C, a potent reducing agent, improves nonheme Fe absorption; and vitamin D improves Ca, P, and Mg transport and absorption. Since carotenoids are fat soluble, the addition of small quantities of fat or oil will enhance bioavailability[4]
- *Presence of absorption inhibitors*: For example, oxalates (i.e. in spinach) and phytates (i.e. in sesame seeds) are rich phosphate anionic chelators of minerals and niacin.[5,6]

A feeding study among humans was carried out in which strawberries, containing 222 μmol of the anthocyanin pelargonidin-3-*O*-glucoside, were consumed with and without 100 mL of double cream.[7] The cream reduces, but not significantly, the plasma C_{max} of the main metabolite, a pelargonidin-*O*-glucuronide. Cream did, however, significantly extend the T_{max} of the glucuronide from 1.1 ± 0.4 h to 2.4 ± 0.5 h. The 0- to 2-h urinary excretion of the glucuronide and smaller quantities of other metabolites were significantly reduced when the strawberries were

eaten with cream but the reverse occurred during the 5- to 8-h excretion period, and overall there was no significance difference in the 0- to 24-h excretion of the metabolites, which was equivalent to $\sim 1\%$ of anthocyanin intake.

- *Interactions with other drugs/foods*, for example, antacids, alcohol, and nicotine; inhibitory interaction effects can be nutritionally advantageous as in the case of dose-appropriate phytosterols that may be an adjunct for lowering the absorption of low-density lipoprotein cholesterol in patients with hyperlipidemia.[8]
- *Interactions* with other foods (e.g. grapefruit juice, pomelo, cranberry juice, brassica vegetables) via enzymatic inhibition (decreased rate of metabolism), for example, grapefruit juice inhibits CYP3A → higher nifedipine concentrations.[9]
- *Diuretics* may deplete vitamin C, folate, Fe, Mg, K, and Zn. Conversely, enteral feeds may interfere with the uptake of certain medications, such as ciprofloxacin, phenytoin, and coumadin.[10]

Supplement factors in bioavailability

Physical properties of a drug (hydrophobicity, pKa, solubility, particle size, and binding cations) represent significant variables, all of which influence bioavailability.

The drug formulation and encapsulation, excipients used, manufacturing methods, immediate release modified release—delayed release, extended release, and sustained release—and dosage all contribute to bioavailability.

Excluding vitamin C, smaller doses of nutrients do appear to increase the percentage of nutrient absorbed. However, a modification of dosage form, surprisingly, does not seem to affect the location of maximum absorption along the gastrointestinal tract. Water-soluble nutrients—both vitamin C and mineral salts—initially appear to be absorbed quite rapidly. In the case of mineral salts, most notably zinc, a delay appears before a second phase of absorption occurs later along the gut. The lipophilic nutrient, β-carotene, appears to be more slowly absorbed, but this is said to reflect the time needed to produce an emulsified or protein-bound form compatible with transport into the serum, thereby facilitating bioavailability. The reduced bioavailability of β-carotene and vitamin E in soft gel form may also be related to competition with the vegetable oil-based vehicle, present in excess, for emulsification by chylomicrons integral to absorption. This is in contrast to the normal expectation of the impact of oil-based delivery in the presence of an emulsifier.[11,12]

Generally speaking, it is probably fair to assert that micronutrients that are intrinsic to food and those that are delivered through supplements have differing bioavailabilities that are mediated via a complex array of processes and interactions. These collectively can be understood in terms of the Absorption, Distribution, Metabolism, and Excretion (ADME) model, which was developed in order to characterize the pharmacokinetics of medications and chemicals in general. Unlike many drugs, micronutrients may not act efficiently in isolation but together with compounds in the food matrix may have synergistic effects.[13]

Bioavailability and the microbiome

It has long been recognized that gut microbes contribute to biosynthesis and bioavailability of vitamins. However, the magnitude of this contribution—the role of microflora in the catabolism of polyphenols and the production of some active metabolites—in healthy and unhealthy patients remains poorly understood.[14] The underpinnings of the magnificently orchestrated chaos of the microbiome are ultimately about bioavailability. The molecular structure of bacterially synthesized vitamins is not always identical to the dietary forms of the vitamins. In fact, several specialized epithelial transporters have been recognized to participate specifically in the absorption of vitamins derived from gut bacteria.[15]

There is a significant literature that focuses on the understanding of the mechanisms and regulation of transport of water-soluble vitamins at the cellular and molecular levels. In addition, the 5′ regulatory regions of the genes that encode a number of the involved transporters have been cloned and characterized in transgenic mice, thus providing insight about the transcriptional regulation of these events. Moreover, there is some understanding of the mechanisms involved in intracellular trafficking and membrane targeting of the carrier proteins and how clinically significant mutations may result in dysfunctional transport.

Finally, the identification in the large intestine of efficient and specialized carrier-mediated systems that are capable of absorbing a number of bacterially synthesized vitamins (thiamin, folate, biotin, riboflavin, pantothenic acid) raises the possibility that this source of vitamins may play a significant role in regulating the normal body homeostasis of these vitamins and, in turn, that of supplemental vitamins. In terms of the microbiome and macronutrients, another interesting and still unresolved dimension is the recycling of intestinal nitrogen and bacterial amino acids to the systemic metabolism.

One additional set of bacteria-related antecedent variables impinging on bioavailability lies with the high inter-individual variability in human subjects observed in some metabolic processes, especially those in which the microflora are involved.[16]

Bioavailability of the polyphenols in terms of classical ADME

Polyphenols (polyhydroxyphenols) are xenobiotics, that is, human physiology does not produce them. They are secondary metabolites of plants, macromolecules of molecular

weights between 500 and 4000 Da, with at least 12 phenolic hydroxyl groups, 5–7 aromatic rings per 1000 Da, and significant binding affinity for proteins. Large conjugated systems of pi electron configurations impart distinctive ultraviolet/visual absorption characteristics. In vitro studies have demonstrated the activity of individual dietary polyphenols as potent free-radical scavengers, but, like so many observations in medical nutrition research, experimental findings need to be supported by in vivo studies with careful attention to appropriate dosing consistent with real-life intake patterns.[17]

Greater than 8000 polyphenolic compounds have been identified in various plant species.[18] All plant phenolic compounds arise from a common intermediate, phenylalanine, or a close precursor, shikimic acid. Polyphenols occur largely in conjugated forms, with one or more sugar residues linked to the hydroxyl groups. Association with other compounds, such as carboxylic and organic acids, amines, lipids and linkage with another phenol, is also common.[19]

Polyphenols have been extensively reviewed.[20] There is a daunting level of complexity and variability in the biochemistry and possible clinical relevance of these compounds, but for the purposes of the present overview, we highlight key points that will in turn support enhanced awareness and meaningful clinical research into the bioavailability and impact of these nutrients.

Polyphenols may be classified into different groups as a function of the number of phenol rings that they contain and on the basis of structural elements that bind these rings to one another. The main classes include phenolic acids, flavonoids, stilbenes, and lignans.

As previously noted, bioavailability appears to differ greatly among the various polyphenols, and the most abundant polyphenols in the human diet are not necessarily those that have the best bioavailability profile.

Prasain et al. reviewed the risks and benefits of dietary phenolics (flavonoids, in this case) as compared with those delivered via supplements.[21] Supplements containing flavonoids and isoflavonoids have been met with criticism, due to adverse endocrine effects likely related to dose and bioavailability.[22] The reasons for this contrast between phenolics derived from supplement versus food are not fully understood but may involve "missing" synergistic effects that accrue with the authentic food matrix or perhaps altered digestion, release, and degradation of the compounds, leading to reduced bioavailability. Matrix release, solubilization, cellular uptake, and biotransformation remain poorly understood. Major aspects that deserve more attention when estimating bioavailability include effects of innovative processing techniques, synergistic effects of mixed/whole diets, factors effecting micelle formation, co-constituents influencing influx and efflux via transporter systems, or altering phase I/II metabolism, as these have often been overlooked or excluded from consideration, in part due to the difficulties to include in their study in vivo or in vitro.[23]

Polyphenols are extensively modified, not only in the small intestine and colon but also in the liver, where most conjugation takes place. Any single polyphenol may generate two or three metabolites though some, that is, quercetin glycosides, may yield as many as 20 metabolites.[24] The flavonol quercetin occurs in fruits and vegetables with a particularly high concentration in onions. Anthocyanins are present in fruit where they are responsible for red, blue, and purple colors. The flavan-3-ols (−)-epicatechin and (+)-catechin are also present in fruits, cocoa, and green tea.

Tissue distribution

D'Archivio et al. underscore the conclusions of Del Rio et al.[17] in observing that the data on the tissue distribution of the polyphenols are derived principally from rodent studies, but differences between the human and animal genomes may also lead to potential problems of extrapolation.[25] Another threat to the validity of animal study is that rodents methylate dietary phenols far more extensively than humans. Moreover, most of the data from humans presented in the literature on the bioavailability refer only to the release of the polyphenols from the food matrix and their consequent absorption (i.e. concentration in the blood or in the urine). The determination of the bioavailability of the polyphenols in target tissues is obviously much more important than the knowledge of their plasma concentrations. Yet another level of methodological difficulty lies in the challenges of choosing the key active metabolite for study, rather than one of the myriad aglycones or their sugar conjugates.[17]

Finally, it cannot be emphasized enough that determination of the actual bioavailability of polyphenol metabolites in tissues is likely much more important than having knowledge of their plasma concentrations.[16] The kinetics of penetration and elimination of polyphenols in the tissues seems underrepresented in the literature and may well represent an emerging research frontier. A caveat on reading the research literature with a critical eye recognize the limitations of in vitro systems, the significant genomic differences between animal models and humans, and be wary of exaggerated or arbitrary and acute dosing in model systems that either significantly under- or overshoot actual chronic exposure in free-living humans eating a typical food matrix ad libitum.

Absorption and metabolism

Polyphenols are extensively modified during absorption. The glycosides may be hydrolyzed to aglycone in the small bowel or in the colon, then end up in the liver via the portal circulation. Transport across gut mucosa probably occurs via active transport mechanisms.[16] Hepatic metabolism promotes conjugation (and solubility/serum availability) via methylation, sulfation, or glucuronidation. This promotion does not mean that polyphenol metabolites are free in

the blood. There is evidence that binding to plasma proteins is the predominant mode of distribution. High affinity for albumin and marked hydrophilicity vary with specific chemical structure.

While solubility is not an issue for most polyphenols, Bohn et al. documented that lipophilic compounds such as carotenoids, phytosterols, and triterpenes require emulsification/micellization prior to uptake.[23] It is further observed that micelle size, shape, constituents, and macronutrient milieu remain understudied and poorly understood. The relevance and specific array of interaction with brush border enzymes, transporters, and subsequently with colonic microflora are also less well understood.

Some polyphenols, that is, quercetin, are able to interact directly with lipid membranes. In fact, at physiologic pH, and at nutritional doses, many polyphenols form hydrogen bonds with the polar head groups of phospholipids at the membrane surface.[26,27]

Other factors influencing bioavailability

Environmental factors

It is conventional wisdom at this point to declare that conditions under which plants are cultivated have an impact on bioavailability of the fruits of harvest. Sun exposure, wavelength of light in artificial environments, pH of the soil, constituents and degree of fertilizer use, degree of ripeness at harvest, and morphological source, that is, stem, leaf, flower, or fruit, all have impacts on character of nutrients and their bioavailability.

Food processing factors

Thermal treatments, homogenization, lyophilization, cooking (boiling, frying, steaming), storage, mechanical treatments, that is, grating, cutting, chopping, slicing, mashing, and juicing (components released from disrupted tissue matrix), may have significant influence on the bioavailability of nutrients from enzyme activation (i.e. polyphenol oxidase and alliinase).

It is worth detailing that the impact of (mild vs. moderate) heat or cooking; thermal and nonthermal processing, and cooking methods can result in staggering variation in the bioavailabilities of the spectrum of polyphenols. At lower level heat, carotenoids decrease but increases with elevated heat.[22]

D'Archivio et al.[17] cite examples of a reduction in total phenolic content with heat, while others report reduced antioxidant activity in beans.[28,29]; however, in contrast, Rocha-Guzman et al. reported a significant increase in antioxidant activity in another species of beans (*Phaseolus vulgaris* L.) cooked at 121°C.[30] Similarly, Khatun et al. observed that total phenolic content and antioxidant activity in other species actually increased following the application of heat.[31] In yet another example, the concentration of hydroxytyrosol, elenolic acid, idecarboxymethyl oleuropein aglycon, and oleuropein aglycon in olive oil after thermal treatment decreased more quickly than other phenolic compounds.[32] Miglio et al. demonstrated that carrots lost only 1.6% their total polyphenols after boiling, while steaming and frying had a significantly greater negative effect at 10.9% and 17.5%, respectively.[33] Again, variation in response to heat across the inventory of phenolics is the rule rather than exception; some compounds contained in olive oil are subject to degradation upon the application of heat during cooking, although this loss among the different phenolics is inconsistent.[28,34,35] In particular, the concentration of hydroxytyrosol in virgin olive oil rapidly decreases after frying. By the end of the first process of frying (10 min at 180°C), hydroxytyrosol decreased by 40%–50% of its original concentration.

Storage affects the content of polyphenols. In general, but with a few notable exceptions, storage of any kind, including refrigeration results in a decrease in phenolic compounds. This is true for most fruits, vegetables, wines, and olive oils.[36–38]

Food-related factors

Studies of bioavailability in the context of a realistic food matrix yield an array of positive or negative effectors and strikingly mixed results. There is enhanced availability of polyphenols in the presence of bovine milk, soy and rice beverages, sugars, ascorbic acid, and fats. However, availability from foods of different sources and in varying combinations yields negative results.[22] For example, studies with raspberry juice and blueberries demonstrated that addition of dairy products showed reduced recovery of anthocyanins.[39] Differences in bioavailability of catechin metabolites from milk versus dark chocolate and chocolate with varying sucrose levels yield similarly disparate results, leading us to respect the peripatetic influence of the food matrix.[40,41]

Host-related factors

Variability of intestinal factors, that is, enzyme activity, transit time, colonic microflora, efflux transporters (P-gp, BCRP, and MRP3), and transporters (SRB-1, CD36, NPC1L1, and ABCG5/G8) all contribute to host-specific intervening variables that influence bioavailability in a given individual and nutritional milieu.[22] Systemic factors, such as gender, age, genetics, comorbidities/pathology, and target tissue uptake, also create a constellation of factors that must be considered.

Conclusion

Even a brief consideration of the enormous constellation of variables that may shape and drive bioavailability makes it abundantly clear that the ADME model is more than a

helpful heuristic. It is the critical algorithmic basis for building a detailed, comprehensive, and modifiable understanding of the bioavailability, efficacy, and safety of difficult to characterize and important components of human nutrition, such as polyphenols. The variability and inconsistency in matrix release, biotransformation, target tissue uptake, and the gestalt of health impact of micronutrients require us first and foremost to return to basics. If medical nutrition is to deserve that descriptor, it must be viewed even more carefully and rigorously than the way we study drugs. Nutrition research conducted properly is in reality at least as complex as pharmacology.

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