WHITE PAPER

Bioavailability of Supplemental Dietary Nutrients: Challenges and Conundrums

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The Point Institute, founded and directed by Thomas Guilliams, Ph.D. is an independent research organization focused on examining and disseminating information about the use of natural therapeutic options for treating and preventing chronic disease. Along with therapies generally defined as lifestyle interventions, the Point Institute specializes in the evidence and application of nutraceuticals (dietary supplements, vitamins, minerals, botanicals, probiotics, etc.) as therapeutic and preventative agents in clinical practice.

Bioavailability of Supplemental Dietary Nutrients: Challenges and Conundrums

While most would argue that nutrients are ideally delivered through a diverse and healthy diet, it is equally arguable that most individuals simply do not ingest an ideal diet that provides an adequate intake of all necessary nutrients. Therefore, the use of supplemental nutrients delivered in capsules, tablets, powders or liquids is routinely used to augment the intake of nutrients which may be lacking in a person's diet. Furthermore, since many individuals choose nutrient supplementation to optimize their nutrient status or for therapeutic reasons (often at doses well above that which is needed for preventing deficiency-related disorders), there are occasions when supplementation occurs at doses not feasibly achieved through ordinary dietary sources. However, since most supplemental nutrients differ from their food-derived counterparts, there is much debate about the comparative benefits of delivering nutrients from different sources and forms.

One of the many facets of this ongoing debate is focused on bioavailability, and ultimately, bio-efficacy. In other words: when nutrients are delivered as mixtures of isolated compounds, outside of their normal food matrices, how does this delivery affect their nutrient function? Since the efficacy of most nutrients is somewhat related to their bioavailability, there is some concern that the different characteristics (i.e., forms, sources, matrix) of the nutrients used for supplementation have different bioavailability and/or bio-efficacy compared to their food-bound equivalents. In some cases, significant technologies are being implemented to increase the bioavailability of certain compounds with inherently low bioavailability (e.g., CoQ_{10}, curcumin, etc.) in an attempt to increase their therapeutic potential. In this paper, we explore the unique challenges and conundrums created by delivering isolated nutrients- focusing mainly on bioavailability. As this review shows, there is no universal "rule of thumb" that can be applied to all nutrients; requiring, instead, that each nutrient or nutrient class be understood on a case-by-case basis.

Bioavailability and Other Notions: Clearing up Confusing Terms

Since our discussion is mostly about differences in bioavailability, it is important that we define, and perhaps even reassess, what is meant by this term. This is especially important in light of how many dietary supplement ingredients are marketed based on their "bioavailability." Most often, the amount of a substance that gets into the blood (when taken orally) is deemed its bioavailability; however this is only the first step (absorption) in delivering an active substance to the target tissues at a dose that allows for a predictable outcome (its bioactivity). Since most nutrients are studied in the same manner as drugs, using traditional models of pharmacokinetics and pharmacodynamics, we first discuss this classic model. However, unlike compounds designed for drug development, many nutrients utilize specialized transporters or metabolic pathways and many nutrients have now been shown to impact human health without ever entering the bloodstream (i.e., their efficacy targets are human or microbial cells in the gut). Therefore, while traditional pharmacokinetics helps us understand the therapeutic potential of some nutrients, these models are often inadequate to define a nutrient's true bioavailability or bio-efficacy.†

† We should note that this paper focuses on nutrients consumed orally (and swallowed). The delivery of nutrients using intravenous, sublingual, transdermal, intranasal or other routes is not discussed here, except to say that these routes are all drug-delivery systems and any nutrient delivered through these routes, regardless of dose, is considered a drug (in the United States, by FDA). As with each nutrient taken orally, these routes affect each nutrient differently and also need to be evaluated on a case-by-case basis.

In the traditional pharmacokinetic model, bioavailability is defined as the amount of active "drug" in circulation after its administration. In this model, intravenous delivery of an active drug provides 100% bioavailability, whereas oral delivery is diminished by many factors including breakdown of the delivery vehicle (capsule/tablet), its solubility, limitations of absorption through the gut wall, metabolism in the gut or enterocyte, etc. However, much more information is necessary to fully understand what happens to a substance when it is consumed orally. From the traditional pharmacokinetic standpoint, this is described by the acronym ADME (Absorption, Distribution, Metabolism and Excretion). As Figure 1 shows, after a substance is administered orally and arrives in the GI tract, the first hurdle for bioactivity is absorption; that is, transport from the gut lumen into the blood. Depending on the substance, a variety of outcomes are possible after absorption including distribution to various tissues (where the substance might be sequestered and accumulate), metabolism to one or more active or inactive compounds, and excretion (typically in the urine or feces). Immediate metabolism by the liver (usually called "first-pass metabolism") is often the fate of a large portion of substances delivered orally, though similar enzymes in the enterocytes often metabolize active substances before they ever enter the blood stream.

When considering all of these steps, a better way to define bioavailability is the amount of bioactive substance(s) reaching the biologically active site(s) within the body. For most drugs, data gathered from cell culture research is often used to predict the concentration of an active compound that might be needed for a clinical outcome (see sidebar on why *in vitro* research often leads us to false predictions about the concentrations needed for a substance's bioactive concentration). Ultimately, delivering the appropriate *bioactivity* without introducing toxicity is the therapeutic goal in delivering any therapeutic substance.

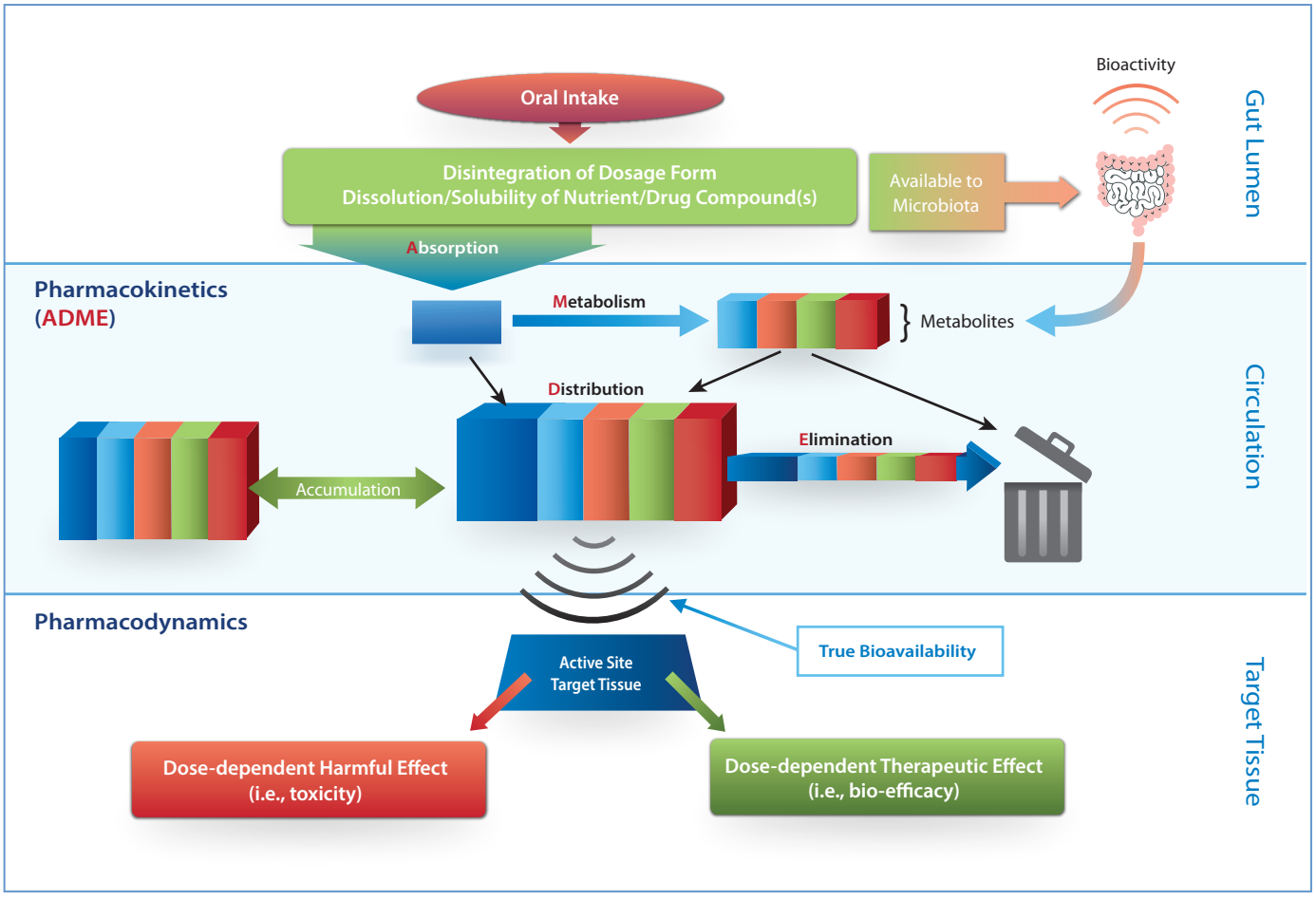

Figure 1: True Bioavailability and the Classic Pharmacokinetic Model. The traditional "ADME" pharmacokinetic model is shown here with several important modifications. First, while absorption from the gut lumen to the serum is considered the first step of the model, it is important to note that many nutrients that "fail to absorb" are available to the gut microbiota allowing significant direct bioactivity, or metabolism of the parent compound (many of these metabolites may be subsequently absorbed). The multi-color bars are used to re-enforce the idea that there are numerous metabolites for each absorbed nutrient that may affect its eventual accumulation, elimination and target tissue bioactivity. While many conflate absorption with bioavailability (or even bioactivity), we believe true bioavailability is only measured when the bioactive compound reaches its target tissue (in the body or in the gut). See text for more details.

Lost in Translation: The Disconnect Between Bench and Bedside

The drug-development process usually begins by screening a myriad of natural and synthetic compounds for a specific bioactivity. This is usually accomplished by choosing a model assay and replicating this assay using an array of compounds. For instance, if you wanted to find out how various synthetic modifications of curcumin would affect the killing of different cancer cells, you would synthesize and purify a number of compounds, then add them to the culture medium of several different kinds of growing cancer cells and determine which compounds could kill each type of cancer cell. This is usually performed with a variety of concentrations of each substance, to determine which compound is the most potent (has the greatest effect at the lowest dose). More recently, screening substances for their ability to alter gene or protein expression in one or more cell type is also common. In some cases, there are no cells involved; the experiment may include only a purified enzyme to test the inhibitory activity of a group of compounds. Regardless of the screening process, the goal is to discover a compound that has a high bioactivity at a relatively low concentration; a combination that will increase the possibility of achieving a high enough bioactivity when taken orally, while limiting potential toxicity.

While this description of drug-discovery is quite simplistic, it will suffice to discuss some of the issues that thwart the effective translation of most substances discovered through this type of screening. First, it should be pointed out that these types of experiments are designed to screen a large number of compounds for their potential benefit, using a limited set of criteria. Often the goal is to assess a previously known drug mechanism (e.g., screening natural compounds for HMG-CoA reductase inhibition) or expression of a known activator (e.g., testing compounds for reduction in NF-kB expression). However, regardless of how sophisticated the screening tool might be, it is equally limited in many important ways.

One important limitation is the fact that few substances interface directly with cells or enzyme systems without also interacting with a myriad of other cells and metabolites. For instance, when a compound is added to the culture media of cancer cell lines growing on plates, these cells are growing in a monoculture with no blood supply, no immune system cells, with no surrounding tissues in a standardized media of nutrients and metabolites. In other words, the environment of the cell or enzyme system is very artificial and, in most cases, cells capable of growing in such culture environments express different genes and produce different metabolites than those same types of cells growing in the body. Imagine how many types of signals are waxing and waning for a normal cell which cannot be mimicked *in vitro* (e.g., hormones, cytokines, circadian changes, nutrient changes, etc.).

Another important limitation related to interpreting these *in vitro* tests involves the compounds themselves, especially when it comes to testing natural compounds. In most cases, the substances that would be ingested (e.g., quercetin, resveratrol, lipoic acid, etc.) are being added directly to the cells or the enzyme assay. However, even if these substances are adequately absorbed orally (though many are not), they rarely do so without being metabolized to other compounds, with differing levels of bioactivity compared to the parent compound. In other words, the compound used for screening the activity *in vitro*, is often different than the compound's metabolite which reaches the tissues when taken orally. This is especially true for phytochemicals, but this principle applies to many essential nutrients as well. Cell culture or animal models that show clear outcomes (positive or negative) are often not seen when translated to human oral consumption. Therefore, when evaluating pre-clinical research of nutrients and natural compounds, it is vital to understand that these results only provide a *potential* clinical bioactivity (pharmacodynamics) while avoiding the many obstacles preventing the tested compound from reaching the target tissue (pharmacokinetics).

Therefore, as we discuss the various ways that supplemental nutrients are delivered, especially when they are modified to alter their inherent absorption characteristics, it is critical to ask whether these modifications alter bioactivity (or toxicity) before assuming that improved absorption improves clinical outcomes.

Essential Vitamins and Minerals

The absorption of vitamins and minerals from foods is influenced by a number of mechanical and chemical steps including food preparation, cooking, chewing, stomach acid, digestive enzymes, bile and a wide range of transporters along the cell surface of enterocytes and colonocytes. Ironically, since most vitamins and minerals in foods are embedded in a matrix, isolated nutrients provided in supplements often absorb

at a higher rate (as a percent of the total amount ingested) compared to their food-bound counterparts. In some cases, this difference is enhanced by the fact that certain foods may contain compounds that diminish the absorption of some other compounds in the food matrix (e.g., phytates inhibit iron and zinc absorption).[1] However, before delving into specific vitamin or mineral examples, there are a few principles that should be considered when attempting to understand the magnitude of nutrient absorption, as well as the research used to compare the absorption and bioavailability of different nutrient forms.

- For many essential nutrients the rate of absorption is affected by the dose of nutrient consumed, where the relative rate of absorption is greatly attenuated above certain doses (i.e. absorption capacity is saturated). This reality is often based upon the relative concentration of nutrient transporters along the gut and the overall surface area of a person's GI tract. For these nutrients, smaller more frequent doses are likely to improve overall nutrient absorption, nutrient status and relative bioavailability. Calcium absorption is a classic example, though many water-soluble vitamins and minerals are influenced by this phenomenon as well.[2]
- The absorption of some nutrients is directly affected by the subject's baseline status for that nutrient.[3] That is, when the status of certain nutrients is adequate, there is a down-regulation of intestinal transporters which limits further nutrient absorption; whereas these same transporters are up-regulated when the nutrient status is low. The fact that many studies do not measure study participant's nutrient status prior to supplementation is a fundamental challenge to interpreting their outcomes.
- While not well studied in most subjects, the absorption of one nutrient may interfere with the absorption or status of another. Perhaps the best characterized example, in humans, is the effect of zinc intake on copper absorption or status (though many other similar relationships are postulated).[4]
- Some water-soluble nutrients, when taken at very high levels, absorb passively and independent of their normal absorption mechanisms. This can be viewed as therapeutically positive (i.e., oral vitamin B_{12}) or as potentially negative (i.e., folic acid), depending on the person and the dose.
- The absorption and/or transport of several vitamins requires conversion to a "free" state. This affects the functional utility of so-called "activated" forms of certain nutrients and, ironically, results in certain "synthetic" nutrients forms absorbing better than their natural counterparts (see details below for pyridoxine-5-phosphate, riboflavin-5-phosphate, folic acid, 5-MTHF and methyl-B_{12}).
- Timing supplemental nutrient intake with meals (or away from meals) may influence the relative absorption of certain nutrients, though the content of the meal itself may increase or nullify this effect. However, the long term benefit of consuming supplemental nutrients when it is most convenient for the consumer (thus improving adherence), may outweigh the benefits of consuming nutrients at specific times in relation to a meal (note: taking some nutrients on an empty stomach can induce nausea in some individuals).

Vitamin Forms: Comparing Bioavailability, Bioequivalence and Bio-efficacy

The absorption of most vitamins, or their provitamin precursors found in foods and supplements, is facilitated by a variety of transporters along the GI tract. For the most part, these transporters are designed to absorb only a "stripped-down" version of each vitamin, from the many different forms found in nature. This means that many vitamin compounds must first be modified before they are absorbed, some of which then go through elaborate modification to their activated forms once in various tissues. Therefore, while many believe that there is a benefit in oral supplementation of "activated" vitamins, it turns out that few of these versions improve either bioavailability or bio-efficacy.

The most straight forward examples of this phenomenon are the phosphorylated forms of vitamin B_6 (pyrodixal-5-phosphate), riboflavin (riboflavin-5-phosphate) and thiamin (thiamin monophosphate).[5] In each case, only the non-phosphorylated versions can be moved through their respective transporters. Therefore, when taken orally the so-called "activated" form requires additional enzymatic processing (de-phosphorylation) in the gut lumen or enterocyte to become bioequivalent to an equimolar amount of its unphosphorylated form. In the body, these vitamins are activated when needed, and deactivated when being transported between various tissues. We should note, however, that all of these forms (whether natural or synthetic) are essentially bioequivalent, though the phosphorylated forms are significantly more expensive to purchase because they require additional processing steps in their commercial synthesis.

Another example are the many different supplemental forms of cobalamin (vitamin B_{12}): methylcobalamin, cyanocobalamin, hydroxycobalamin and adenosylcobalamin. Ironically, most of what we know about vitamin B_{12}

Dietary Folates vs Synthetic Folic Acid and 5-MTHF

Folate is the generic term for naturally-occurring food folates, folic acid, folinic acid and 5-MTHF. Plant-derived dietary folates exist mostly in polyglutamate forms, while commercially available folates, such as folic acid, folinic acid and 5-MTHF are monoglutamates. Polyglutamate folates from the diet must first be hydrolyzed to their monoglutamate forms by the action of folate hydrolase before being absorbed. Thus, when establishing food recommendations, the bioavailability of food-derived folate is commonly estimated at 50% of folic acid (already a monoglutamate). Monoglutamate forms that are not fully reduced (e.g., folic acid) or methylated (e.g., both folic acid and folinic acid) prior to absorption, will be reduced and methylated to form 5-MTHF within the mucosa or liver prior to circulation. Once in the target cell, additional glutamate molecules are added to 5-MTHF, to form a polyglutamate once again.

Therefore, the current recommended dietary allowance (RDA) in the United States is measured in Dietary Folate Equivalents (DFE, see below), which are now mirrored in the label-claim daily value guidelines on supplements and foods. Use of the DFE reflects a higher bioavailability of synthetic folates found in supplements and fortified foods compared to that of naturally occurring food folates.

- One mcg of food-derived folate provides one mcg of DFE
- One mcg of folic acid or 5-MTHF taken with meals or within a fortified food provides 1.7 mcg of DFE
- One mcg of folic acid or 5-MTHF (in a supplement) taken on an empty stomach provides two mcg of DFE

However, genetic polymorphisms in some individuals can alter the bioequivalence of the two major folate compounds used in supplements (i.e., folic acid and 5-MTHF). While the details of these differences are beyond the scope of this paper, we should note that absorption of these two molecules is still very similar, even in subjects with multiple polymorphisms in the *MTHFR* gene. Research generally shows that supplemental folic acid, which must be fully reduced and methylated, is often less capable of increasing serum 5-MTHF levels in *MTHFR* 677TT individuals (i.e., homozygous for the genetic variant) compared to 677CC individuals (i.e., wildtype).[1] However, over time, these differences may not be highly significant since one study showed that after 13 weeks of folate treatment (using either a folate-rich diet, folic acid, or 5-MTHF) equivalent to 200 mcg of folic acid, each folate form was equally capable of RBC folate benefits and homocysteine lowering in subjects (Italian) with moderate hyperhomocysteinemia (mean baseline Hcy 14.1 mmol/L).[2]

Nonetheless, beyond these bioavailability and homocysteine-lowering differences, other issues may be important when comparing folic acid versus 5-MTHF supplementation. The first is that high-dose folic acid supplementation (> 5 mg/day) results in an increase in serum levels of unmetabolized folic acid (due to passive absorption at higher levels).[3] This is likely due to overwhelming the capacity of the dihydrofolate reductase (DHFR) enzymes in both the intestine and liver, rather than specific deficiencies in MTHFR enzymes as many believe, though consuming equimolar amounts of folic acid with vitamins B_{12} and B_6 reduces this phenomenon suggesting folate recycling may be involved.[4] There is growing evidence that unmetabolized folic acid may lead to negative outcomes, though more research is needed. However, we now advise that supplementation above 5 mg/day of folic acid, when warranted, should be done with caution or substituted with 5-MTHF supplementation to avoid this phenomenon. Additionally, it is often cited that high doses of folic acid can mask (not cause) an underlying vitamin B_{12} deficiency; supplementation with 5-MTHF appears to be less likely to have this consequence, though this has not been confirmed by rigorous clinical trials. Therefore, it is routinely recommended that vitamin B_{12} supplementation be added during high dose folate/folic acid supplementation therapy.

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supplementation (and metabolism) comes from the use of cyanocobalamin, which was the only form commercially available for many decades. However, now that all forms of cobalamin are commercially available, there is much confusion about the potential therapeutic differences between these forms and the bioavailability differences between each form. Since methylcobalamin and adenosylcobalamin are the active forms, some have even speculated that supplementation of both compounds would be needed for a person to obtain an ideal vitamin B_{12} status. However, mechanistic studies and clinical outcomes do not suggest that these forms are greatly differentiated when consumed orally by humans.[6] In fact, oral supplementation of cyanocobalamin has been shown to readily reduce homocysteine (a reaction requiring methylcobalamin) as well as MMA (a reaction requiring adenosylcobalamin).[7,8] This is because the enzymes that activate vitamin B_{12}, are agnostic as to the starting molecule's configuration; removing and replacing the active group regardless of its initial ligand.[9] In other words, the first step is the removal of the ligand (i.e., methyl, cyano, hydroxy, adenosyl) from vitamin B_{12}, even if it is the same ligand that will be added in the second step. Therefore, except for extremely rare genetic polymorphisms, there does not appear to be any therapeutic differences between these compounds when taken orally (i.e., they are all bioequivalent).

Mineral Forms and Bioavailability

There is much debate about the relative absorption of minerals based upon their form, though there is limited human research on this subject. However, unlike vitamin molecules which are synthesized by some biological organisms, minerals cannot be synthesized per se; they simply move between the soil, water, air, plants and animals. Nonetheless, mineral compounds used for food fortification or dietary supplementation are almost always modified (and different) than those encountered in nature. The low concentrations of dissolved minerals in water are primarily in their ionic form (e.g., Ca^{2+}), while food forms of minerals (especially those from plants) are mostly bound within complexes of proteins or other organic compounds.

On the other hand, minerals used for food fortification and supplementation are typically purified mineral salts or organic complexes; mostly created by reacting concentrated mineral sources with other ingredients. For instance, calcium carbonate is a naturally-occurring salt found in limestone, chalk, coral and seashells which can be used directly in supplements; however, it is often dissolved and combined with an organic acid (e.g., ascorbic acid, citric acid) and precipitated to make a new calcium salt (e.g., calcium ascorbate, calcium citrate). These mineral salts can differ in many important characteristics — percent of mineral content, solubility, pH, bioavailability — differences that may affect their clinical effectiveness. Unfortunately, dietary guideline recommendations for mineral intake do not distinguish the source of the mineral, nor are they standardized for the different bioavailability of various mineral compounds.

Furthermore, unscrupulous ingredient suppliers often misrepresent some mineral blends as new "reacted" compounds when they are merely blended powders. When commercial sources of supplemental mineral compounds are created by reacting two ingredients, such as magnesium oxide and citric acid, a new compound is formed upon precipitation: magnesium citrate. Made correctly, the finished materials should have little unreacted magnesium oxide or citric acid present. However, it is not uncommon for ingredient manufacturers to simply blend these two ingredients as dry powders, while still labeling the mixture (incorrectly) as magnesium citrate. The problem is the unreacted blend, a much cheaper final product, does not have the properties of the truly reacted new compound (e.g., pH, solubility, bioavailability, etc.). Unfortunately, since regulators are more concerned about the accuracy of the elemental mineral claim, little focus in product testing is placed on distinguishing the precise makeup of the mineral compound.

Why does this matter? The bioavailability and mineral content of compounds often differ from one another. A particular example is the vast difference between the relative bioavailability of equivalent levels of magnesium found as magnesium oxide and magnesium citrate. While magnesium citrate has a lower mineral content, a truly reacted compound allows for much higher relative magnesium bioavailability compared to magnesium oxide.[10] However, when the high mineral content of magnesium oxide is diluted by being blended with, rather than reacting with, citric acid, this poorly absorbed form is made worse by merely diluting its mineral content without increasing its bioavailability.

A further category that requires distinction is that which defines a mineral chelate. Technically, a chelate is defined as an organic compound that forms two or more coordinated bonds with a central metal ion (chelate comes from the Latin word for "claw"). There is, however, no regulatory specification for mineral chelates, especially as it pertains to their stability when taken orally by humans. Most true chelates are minerals bonded to glycine in a 1:2 ratio (e.g., magnesium bisglycinate), though lysine and arginine are sometimes used. Amino acid chelates can, if manufactured properly, enable absorption of the attached mineral through the pathway normally used for amino acids, allowing for the release of the mineral once in circulation. By avoiding the standard transport mechanisms for ionic minerals, it is believed that amino acid mineral chelates may also avoid much of the natural competition between mineral absorption observed when high levels of mineral salts are used. These amino acid chelates may also be associated

with less GI discomfort.[11] Combining amino acid chelated minerals with mineral salts or organic complexes may help maximize different pathways for mineral absorption, especially when high doses of calcium or magnesium are warranted for therapeutic reasons.†

Special Nutrients, Unique Challenges

There are a number of ingredients found in dietary supplements that may be described as special or functionally-essential nutrients, some of which are found in only small quantities in the diet. These include ingredients such as coenzyme-Q_{10} (CoQ_{10}), chondroitin sulfate, fish oil (EPA and DHA), glutathione, lutein, or lipoic acid. Each of these ingredients present a unique bioavailability challenge when used as a dietary supplement. In some cases, like CoQ_{10}, when we attempt to deliver a much higher dose of a fat-soluble compound than the body is accustomed to, solubility issues prevent absorption. In the case of CoQ_{10}, this challenge has been solved by various technologies designed to improve solubility.[12] On the other hand, the absorption of some ingredients like chondroitin sulfate are influenced by the molecular size and other properties of the polymers.[13] When chondroitin sulfate is specifically processed to deliver consistently smaller molecular weight particles, it has consistently higher bioavailability than larger, poorly processed particles. Again, since most of these ingredients are unique, the data must be understood on a case-by-case basis. Below we discuss two of these unique challenges.

EPA and DHA from Marine Sources

When marine omega-3 fatty acids are harvested from their source, they are typically in the form of triglycerides (TG), phospholipids (PL), or free fatty acids (FFA) and are relatively low in total EPA and DHA ($\leq$ 30%). When consuming fish or unconcentrated fish oil (i.e., fish body oil or cod liver oil), these fatty acids are in the TG form, as they are in most plant and animal sources of fat. However, since the recommended doses of EPA and DHA are often difficult to consume using unconcentrated oils, several steps can be used to increase the EPA and DHA concentration of a product while increasing the purity of the fatty acids delivered. The EPA and DHA fatty acids can be removed from their glycerol backbone and separated from other fatty acids (via hydrolysis and distillation). These fatty acids are then concentrated as ethyl esters (EE) of EPA and DHA. These concentrated fatty acids can be re-attached to a glycerol backbone to form re-esterified TG (rTG) molecules which contain a much higher concentration of EPA and DHA compared to the original TG molecule. These two forms of concentrated fish oil (i.e., EE and rTG) are the most common sources used in clinical trials and are often recommended by physicians (as dietary supplements or pharmaceuticals).

The efficacy of omega-3 fatty acid therapy is significantly affected by tissue availability, particularly its ability to increase a person's red blood cell (RBC) EPA and DHA (i.e., the omega-3 index), which is affected by its initial bioavailability.[14] Therefore, numerous studies have been performed to compare short- and long-term bioavailability in human subjects using omega-3 fatty acids from different sources and in different molecular forms. Since the initial production and use of ethyl ester (EE) forms of omega-3 fatty acids, many have questioned the potential difference in bioavailability of these forms compared to other natural fatty acid forms. The early studies were small, but these data revealed either a slightly reduced bioavailability of the EE forms (compared to TG forms) in the absence of additional dietary fat or a statistically similar bioavailability between EE and TG forms. More recently, several larger and better-designed studies have shown a superior bioavailability of rTG forms over EE forms.

One of the largest studies performed to date compared similar doses of EPA and DHA using five different forms: unconcentrated triglycerides (which the researchers called fish body oil-FBO), cod liver oil (CLO-similar TG form as FBO), rTG, EE, or FFA, along with a "placebo" of corn oil (CO). In this study, 72 subjects were randomly assigned 3.3 grams per day of an EPA+DHA blend as capsules for two weeks.[15] Serum fatty acids (combined serum TG, PL, and cholesterol esters) were analyzed at baseline and after two weeks. In these subjects, the absorption of EPA+DHA from re-esterified triglycerides (rTG) was superior (+24%) when compared with natural fish oil (FBO or CLO), whereas the absorption from ethyl esters (EE) was inferior (-27%) to natural TG and nearly 70% less than rTG. Concerning the EE form, studies have shown decreased lipase enzymatic activity when EE substrates are used, perhaps accounting for their decreased absorption when consumed away from a meal containing fat.[16]

Ultimately, it is critical to know whether these differences in absorption over two weeks might translate into long-term differences in fatty acid incorporation into important tissues (e.g., RBC or cardiovascular tissues) and whether these differences can be measured in a clinically meaningful outcome (e.g., reductions in TG). These sorts of studies have been carried out by researchers in Germany, who looked at the incorporation of EPA and DHA into red blood cell membranes, commonly referred to as the omega-3 index,

† The data comparing the human bioavailability differences of specific minerals forms, where available, are discussed in each mineral's respective monograph in our Standard Roadmap: *Supplementing Dietary Nutrients- A Guide for Healthcare Professionals* (Point Institute, 2020).

when individuals consumed either EE or rTG forms of fish oil.[17] One particular study included 150 hyperlipidemic subjects who were also taking statin drugs. Subjects were given soft gelatin capsules containing EPA (1,008 mg) and DHA (672 mg) daily as either rTG or EE forms (CO used in placebo group) and were followed for six months. Subjects consuming the rTG form had, on average, a statistically higher omega-3 index than those consuming the EE form after three months, which was maintained after six months of daily intake. In a separate publication, the lipid-lowering effects of these two therapies were also reported.[18] While both the EE and rTG forms reduced serum TG levels in these patients compared to placebo, the change resulting from rTG was nearly double that of the EE form (-18.7% vs -9.4%). The only therapy to reach a statistically significant decrease from baseline was rTG therapy.

In the past decade, the market has been flooded with information about the use of, and purported superiority of, omega-3 fatty acids from krill.[19] These claims have primarily come from two properties of krill oil: that it is composed mostly of phospholipids (PL – as opposed to TG) and that it contains trace levels of astaxanthin, a bioactive carotenoid with antioxidant properties. Additionally, some studies have suggested these properties, particularly the PL nature of the fatty acids, account for superior bioavailability compared to fish oil. In general, only short-term and limited comparisons are available to ascertain the relative bioavailability of krill oil versus fish oil, a research question complicated by krill oil's very low concentration of EPA and DHA. One group studied the difference between the use of krill oil and menhaden oil (FBO, natural TG) or placebo (olive oil) in their ability to alter plasma fatty acids when consumed by overweight and obese subjects (N = 76).[20] Compared to olive oil, both the krill and menhaden oil significantly increased the EPA and DHA levels of the subjects: krill increased EPA by 89% and DHA by 23%; menhaden increased EPA by 81% and DHA by 45%. These data suggest that the bioavailability of EPA and DHA from krill and unconcentrated menhaden oil are similar.

The second study often cited was a seven-week study comparing the change in plasma fatty acids in subjects with "normal or slightly elevated" lipids when given either krill or fish oil.[21] This study compared six capsules of krill oil, providing 543 mg of EPA+DHA, and three capsules of fish oil (unspecified form), providing 864 mg of EPA+DHA. Compared to control subjects (unsupplemented), both krill and fish oil consumption was able to statistically increase EPA and DHA. However, while the average increase in EPA and DHA was slightly higher in the fish oil group, the difference between the groups was not statistically significant. We conclude, as many others have, that the bioavailability of EPA and DHA from krill is not superior to that of fish oil TG or rTG forms and the low concentration of EPA and DHA from krill make it an especially uneconomical way to deliver these compounds.[22]

Lipoic Acid

Alpha-lipoic acid (LA) is a common dietary supplement ingredient, used in many positive clinical trials for its pleiotropic antioxidant potential. However, these studies use doses that could never be achieved with any known dietary source (usually only a few mcg/gm are found in the highest food sources such as organ meats).[23] Instead, supplementation of LA is done using synthetically-derived bio equivalent compounds. Supplemental lipoic acid is derived from chemical synthesis, starting from a modified eight-carbon fatty acid (6,8-Dichloro-ethylcaprylate). This process produces a 50:50 (racemic) mixture of the two stereoisomers of alpha-lipoic acid, *R* and *S*. Nearly every human clinical trial has used this racemic (*RAC*) lipoic acid—both intravenous and oral dosing. Since about 2001, *R*-lipoic acid (potassium and sodium salt forms) has been available and sold as a dietary supplement ingredient in the U.S. While the *R*-isomer is the form found in nature, this supplement ingredient is produced by a complex process that separates the *R* and *S* forms after chemical synthesis. Although animal and cell culture studies suggest the *R*-isomer may have some biological differences when compared to *RAC* alpha-lipoic acid, human clinical studies have yet to confirm these results.

To date, only small pilot trials have been done to test the pharmacokinetic and clinical differences between *R*-lipoic acid and *RAC*-lipoic acid in humans.[24] These studies reveal that there may be large individual differences, as well as age and sex-dependent differences in *R* and *RAC* lipoic acid bioavailability. In any event, these studies are typically performed using high single doses (500 mg) and have not been done with long-term dosing of each ingredient/isomer or with any meaningful clinical endpoints. Since *in vitro* and animal studies suggest many, though not all, of the antioxidant and cell-signaling functions of *S*-lipoic acid (or *RAC*) are similar to the *R*-isomer, it is possible that the *RAC*-form may function clinically similar to *R*-lipoic acid. Regardless, since there are virtually no human clinical studies performed using *R*-lipoic acid to evaluate, and there are numerous studies to help guide clear recommendations for use of *RAC* alpha-lipoic acid, we do not recommend the more expensive *R*-lipoic acid for supplementation or therapeutic intervention.

LA has a relatively short half-life, estimated to be about 30 minutes.[25] Lipoic acid supplementation may be best taken away from food in light of the results of a pharmacokinetic study performed in twelve healthy volunteers where 600 mg

racemic LA administered with food (863 kcal meal) resulted in a reduced $AUC_{0\text{-}t(last)}$ and C_{max} of both LA enantiomers compared to when racemic LA (600 mg) was given in the fasted state.[26] Due to their poor pharmacokinetic performance, time-release forms of LA are not recommended.†

The Phytonutrient Bioavailability Conundrum

A wide range of concentrated and highly purified phytonutrient compounds are now commonly used in dietary supplements. Most of these compounds, such as resveratrol, berberine, curcumin, quercetin, etc., are incorporated into various products designed to promote a therapeutic result. Because many of these phytonutrient compounds are available as isolated and concentrated powders, they have been tested in a battery of pre-clinical studies, not dissimilar to drug-discovery research. However, while numerous bioactivities have been linked to many phytonutrient compounds in pre-clinical studies, these promising results have often been difficult to replicate in human clinical trials.[27] While there are many potential reasons for this lack of clinical translation, the low oral bioavailability of many phytochemicals is often singled out as a primary culprit. Therefore, a significant amount of research has focused on ways to increase the oral bioavailability of concentrated phytochemicals, with the intent of increasing their efficacy. Ironically, while these efforts have often resulted in an increase in the oral absorption of these compounds, little evidence has yet materialized that this has resulted in greater bioactivity in human subjects. Here we use the example of curcumin from turmeric to illustrate this conundrum.

Curcuminoids from Turmeric: A Precautionary Tale

Despite the many historical medicinal uses of turmeric root, including the identification of many bioactive components in turmeric, the research focus over the past few decades has been almost exclusively centered on one group of phytochemical compounds, its curcuminoids.[16] Purified and concentrated extracts of one or more curcuminoids (often 95% or greater) are commonly used in dietary supplements, functional foods, and clinical research trials across the globe (over 8,000 citations in PubMed include "curcumin" it their title!). However, despite its popularity and hopeful research, the clinical results using curcumin in humans are often greatly muted compared to the promising results from *in vitro* research and mechanistic studies. The reason for this disparity is almost universally attributed to curcumin's poor absorption/bioavailability, for which the creation of a variety of bioavailability-enhanced delivery forms has been postulated as the logical solution. However, despite the claims being made about these modified curcumin ingredients (see below), their increased absorption has not yet been demonstrated to significantly increase clinical efficacy in human clinical trials.[28] While this reality has frustrated many researchers, recent studies suggest a reason for this limitation, a phenomenon likely shared by many other botanicals.

Drug-like pharmacokinetic studies of curcumin in humans generally show a very low recovery of curcumin in the serum after oral intake. One study indicated that the amount of free curcumin in human plasma after intake of 3.6 – 12 g curcumin for a week or longer was below 25 nM (for comparison, concentrations typically used in *in vitro* studies mostly range from 1 – 80 mM).[29] The reasons for this low recovery are many, and include processes that affect the intestinal absorption of curcuminoids and several different metabolic steps that occur in the gut lumen, within the enterocytes, in the liver and within target tissues.[30] Curcuminoids undergo extensive metabolism during and after ingestion.[30] Bioreduction of curcuminoids through phase I metabolism forms major products like tetrahydrocurcumin and hexahydrocurcumin. Further, curcumin and its reduced forms are extensively conjugated through phase II glucuronidation and sulfation; leading to formation of conjugated metabolites like curcumin glucuronide, curcumin sulfate, curcumin sulfate-glucuronide, dihydrocurcumin-glucuronide and tetrahydrocurcumin-glucuronide, etc. While some have speculated biological activity for a few of these metabolites, the *in vitro* assessment of the biological activity of the phase II metabolites of curcumin has demonstrated that predominant serum metabolites, like curcumin glucuronides, do not possess significant bioactivity compared to free curcumin. [31,32-34] Additionally, while small curcumin metabolites that have been isolated from *in vitro* studies (such as vanillin and ferulic acid and their derivatives) have been shown to have potential therapeutic effects, little is known about their formation, pharmacokinetics and bioactivity in animals or humans after oral ingestion of curcuminoids.[35,36]

A variety of technologies have been designed to resolve the problem of curcumin's low bioavailability and metabolic inactivation after oral ingestion.[37-41] These technologies include the mixture of agents designed to prevent the efflux and metabolism of curcumin in the enterocyte (piperine) and a range of drug delivery systems such as nanoparticles, liposomes, micelles or phospholipid complexes; a great number of which are commercially available to consumers and researchers. Indeed, both animal and human clinical trials confirm an increase in serum levels of total curcuminoids with

† For our critique of time-release lipoic acid preparations: see our whitepaper online at http://www.pointinstitute.org/wp-content/uploads/2012/10/Time-release-lipoic-acidpaper.pdf.

most commercially available "enhanced" forms of curcumin, when compared to the unmodified curcumin (i.e., 95% curcuminoid).[42] We should note that these increases are almost always measured as the difference of the serum levels (area under the curve, AUC) over a specified time after ingestion, and usually includes all curcumin-related compounds (i.e., including the inactive glucuronide and sulfate conjugates). Free curcumin levels, when reported at all, are significantly lower in their respective differences compared to the total.[39-41,43,44]

As it turns out, this difference is likely to be very important in explaining why the dramatic increases in serum levels of curcumin that result from using these enhanced forms of curcumin have yet to result in efficacy differences that mirror these same increases (if they increase their efficacy at all). In other words, the vast majority of the increase in curcumin levels in the blood after ingestion of these enhanced forms is conjugated (as a glucuronide or sulfide) and, most likely, has little therapeutic benefit. However, what makes this difficult to discern when reading these publications is the fact that these data are typically reported as total curcumin, total curcuminoids or just curcumin. One needs to dig into the materials and methods section of these publications to discover that the serum samples are treated with glucuronidase and sulfatase enzymes, effectively converting all the compounds to "free" curcumin.[45] Recently, this practice has been scrutinized as an intentional way to inflate the purported benefits of these ingredients.[46] Therefore, while large increases in serum total curcumin are realized by using a variety of enhanced-forms of curcuminoids, the increase in bioactive curcumin reaching tissues is likely to be very limited since the majority (~95%+) of the absorbed curcumin is in the conjugated forms. Thus, we conclude that these enhanced forms of curcumin increase *absorption*, but it is misleading to say that these compounds have improved *bioavailability* (i.e., tissue availability of the bioactive form).

Therefore, it is not surprising that while nearly all the enhanced forms of curcumin have been compared (head-to-head) against 95% curcumin in measures of absorption (often incorrectly called bioavailability), there are no published studies which compare these enhanced forms with 95% curcumin using a clinical outcome (i.e., therapeutic effect).[47-52] The lack of interest in this question (or the lack of published data to answer it) is problematic, especially since the primary marketing strategy in the sale of these new ingredients implies greater efficacy at lower doses. Even without direct head-to-head studies, those who have objectively assessed the clinical effects of both enhanced and unenhanced curcumins conclude that they have similar efficacy in human subjects. In fact, one review article on the subject attempts to tackle this conundrum: "*The collected outcomes raise an open question: why significantly improved bioavailability of curcumin does not produce improved pharmacological efficacy….? Here, we attempt to explain the reason that enhanced bioavailability of curcumin is not associated with improved pharmacological efficacy.*"[28]

Another issue that may affect both bioavailability and efficacy, is the fact that the absorption of certain phytochemicals (in this case curcuminoids) diminishes as they become more concentrated and isolated from the other phytonutrient components of the parent plant (in this case turmeric root).[†] In fact, studies show that the relative absorption of curcuminoids is much higher when consumed as turmeric root powder (or whole extract), than when it is consumed as a near purified compound.[53] Ingredient suppliers are leveraging this knowledge and implementing specially designed technologies to extract different active components from turmeric before combining them back together, allowing for the delivery of numerous active ingredients while also improving the bioavailability of the curcuminoids.[54],[37] These types of products are just now being tested for their efficacy in many clinical trials, with promising results.[55]

Gut Microbiota and Phytonutrient Bio-Efficacy

The human gut microbiota is now considered to influence nearly every aspect of human metabolism and health; and is also recognized as an important mediator of phytonutrient therapeutic activities.[56,57] In fact, since many bioactive phytonutrients have naturally low bioavailability/absorption, resulting in relatively high intestinal concentrations after oral ingestion, researchers have begun to consider the gut and its microbiota as the primary target of phytonutrients like curcumin.[58-60] Indeed, during the past several years scientific advances have suggested a strong bidirectional interconnection between the human gut microbiota and curcumin; whereby curcumin metabolism is influenced by certain gut microbiota and curcumin metabolites modulate the function and therapeutic activities of certain gut microbes.[61] In addition, neurohormonal signaling from the gut (e.g., gut/brain, etc.) appear to be modulated by curcumin administration. Therefore, these emerging studies may help to explain some of curcumin's systemic pharmacological activities and mechanisms of action despite its low systemic bioavailability.

The Trend Beyond Curcumin

While it is difficult to generalize the lessons learned from curcumin to all other phytochemical compounds (though many of these same challenges have already been seen with

[†] It is also important to note that turmeric has many other non-curcuminoid active components which contribute to its bioactivity. The overwhelming focus on purified curcumin products is slowly giving way to re-exploring curcuminoids in the context of other turmeric-derived active ingredients with promising outcomes.

other compounds), the trend to alter the absorption of low-bioavailable phytonutrients with a goal to improve their efficacy continues unabated. It is important for the clinician to be aware of these challenges, and to scrutinize the marketing claims for products claiming greater efficacy (or similar efficacy at greatly reduced doses) based on purported greater bioavailability. Often, these efficacy claims are extrapolations from studies designed only to measure absorption (not bioavailability), for which comparative efficacy studies have never been performed. In most cases, these ingredients have yet to prove an efficacy improvement that offsets their increased cost (i.e., an ingredient that costs twice as much should have, at minimum, twice the proven efficacy, independent of its absorption differences). Not surprisingly, some studies are finding that the relative absorption of some phytonutrients are improved when they are within their original botanical matrix or are influenced by the person's microbiota. Finally, while we believe new research and technologies will continue to unlock new and exciting therapeutic bioactivities from botanicals, these newly modified botanical ingredients must be scrutinized as new agents and they must prove to be safe, therapeutically-superior, and a cost-effective alternative for the ingredients they claim to replace.

Conclusion

When attempting to fill the nutrient gap between what a person is consuming in their diet and what they need to maintain or rebuild their metabolic reserves, it is important to know whether those nutrients are absorbing and getting to the necessary tissues for maximum benefit. It is clear that supplemental nutrients can often be delivered using a range of different forms, some of which have significantly different bioavailability in some subjects. However, the marketing of dietary supplement ingredients often exploits subtle differences in ingredients, conflating absorption with bioavailability (and bio-efficacy). As we have shown, most of these differences must be understood on a case-by-case basis, and sometimes only affect certain subjects based on their genetic profile or baseline nutrient status. The use of supplemental dietary nutrients can be an important part of maintaining and rebuilding nutrient reserves, and the foundation for building resilience against chronic diseases. While bioavailability differences can, indeed, make one nutrient form a better choice than another, these claims should always be investigated thoroughly before being taken at face value. Understanding and appropriately leveraging those nutrients with proven advantages in efficacy should be the priority for all those who use or recommend the use of supplemental nutrients.

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