An Update: Supplementing with the Right Dietary Nutrients

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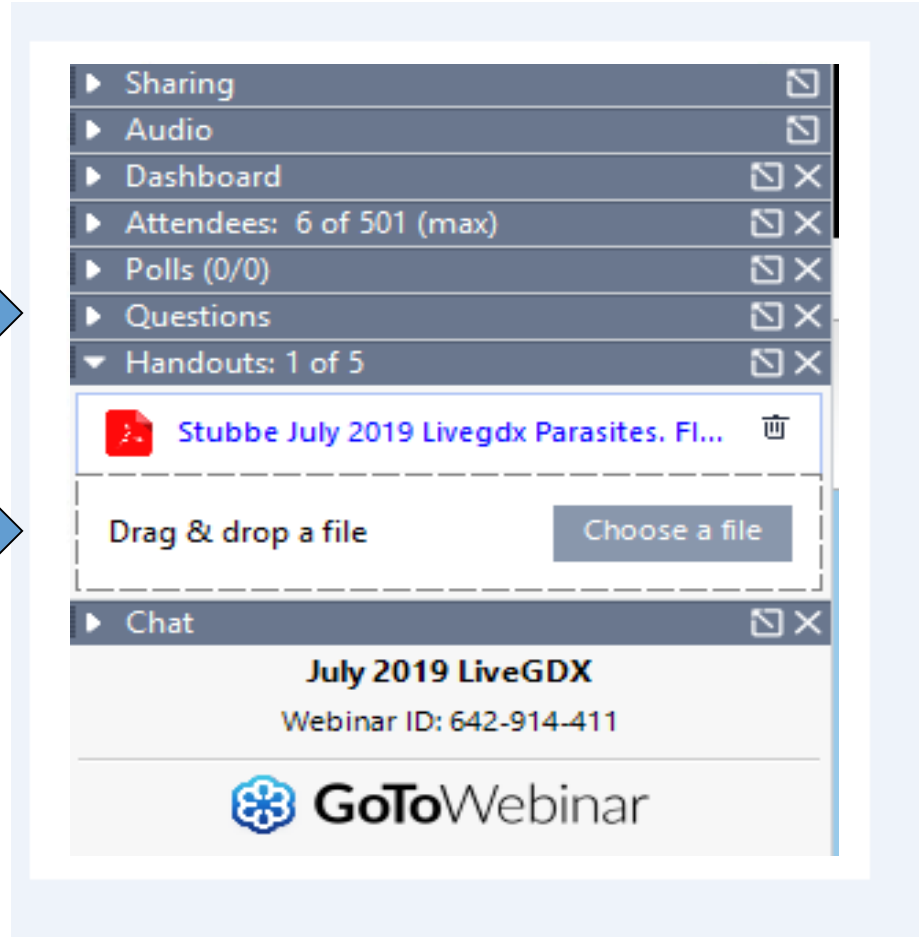

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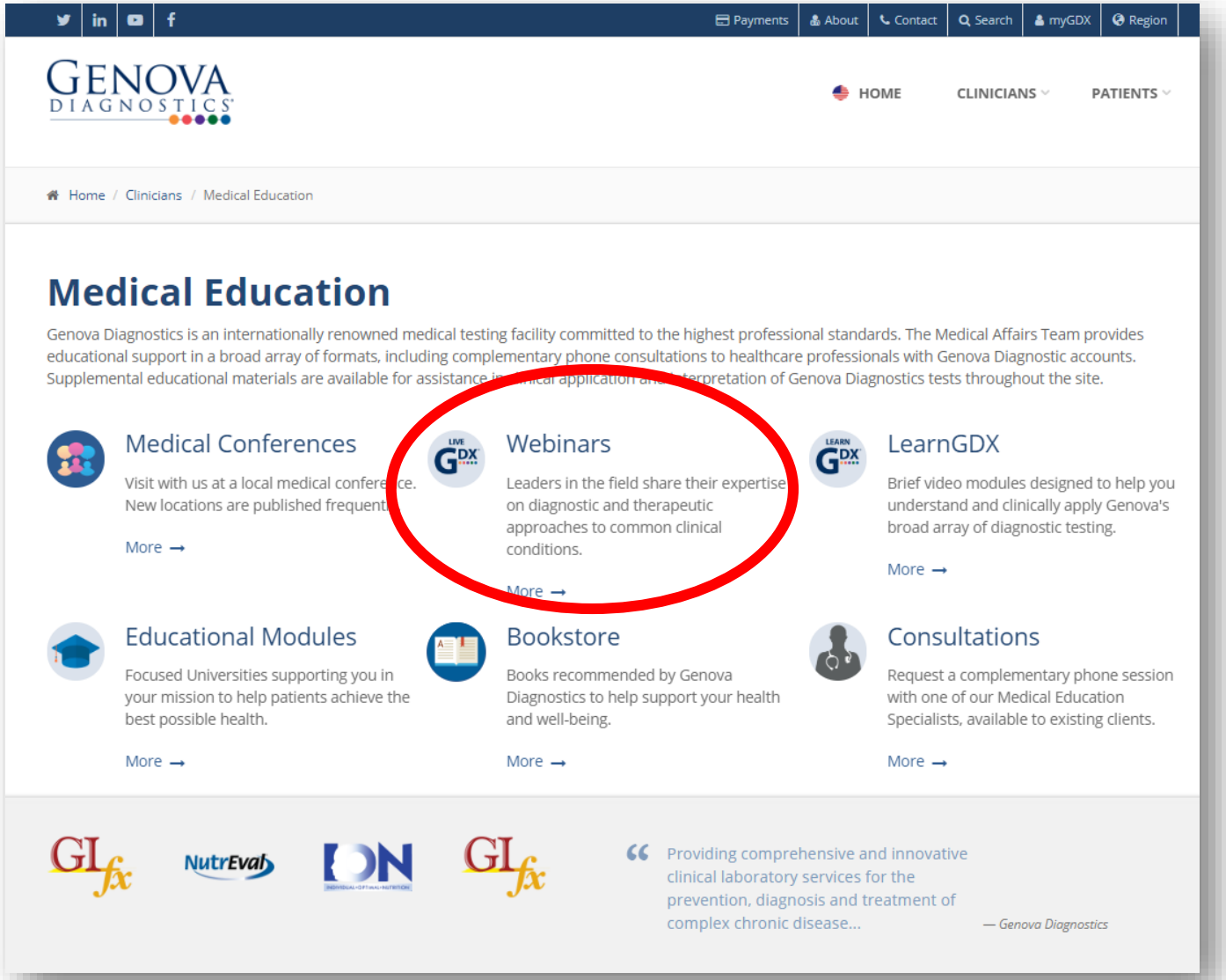

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Objectives for This Presentation

- Help clinicians understand practical differences between "natural" and "synthetic" vitamin compounds and distinguish when those differences matter clinically
- Clear up confusion about many marketing terms used to promote vitamins that are often false or misleading
- Discuss why some bioavailability changes of nutrients (including phytonutrients) alter their efficacy, while others do not

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Foods vs Supplements
Is this a fair fight?

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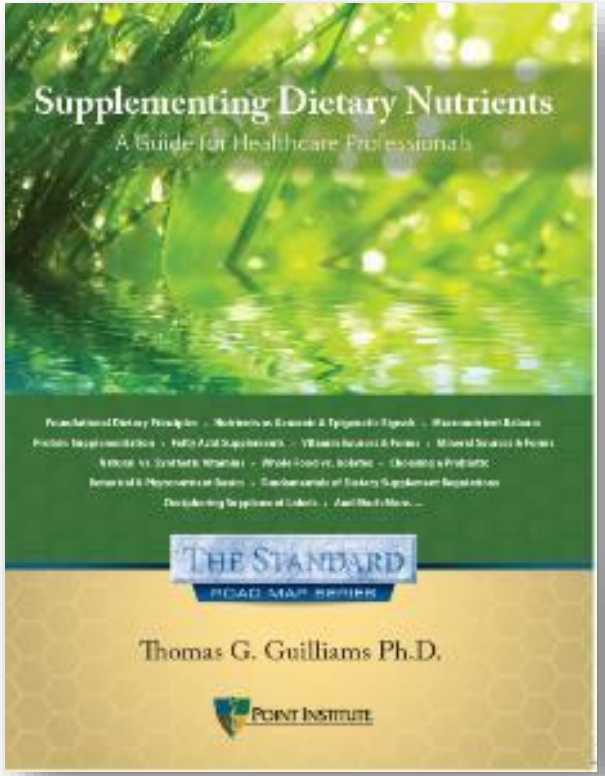
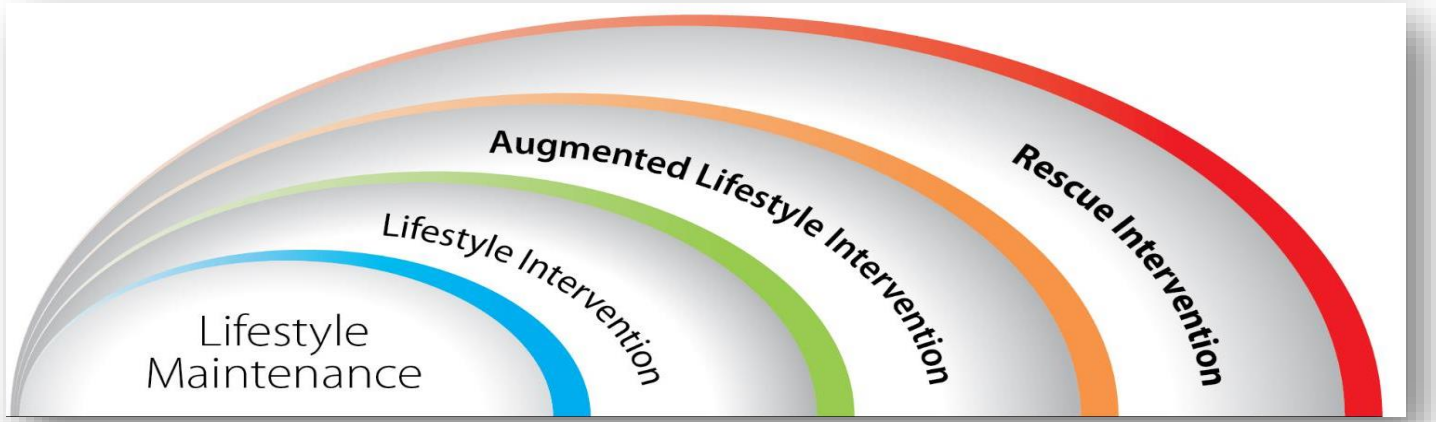
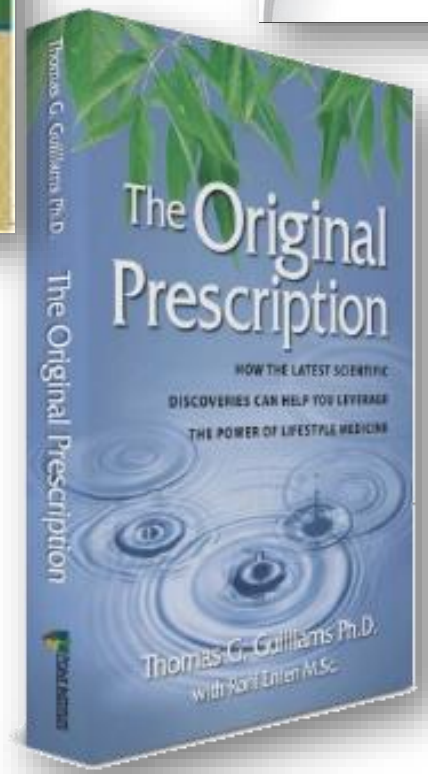
The Basic Premise of Nutrient Supplementation

- Nutrients delivered outside of their food matrices differ from those within natural food matrices (this can be an advantage or disadvantage depending on nutrient, food or subject)
- Most dietary supplement products include additional ingredients that allow for quality and dose control- these can be deemed “unnatural” by some
- Bottom line: Nearly all “supplementation” requires some “compromise” and is not purely “natural”







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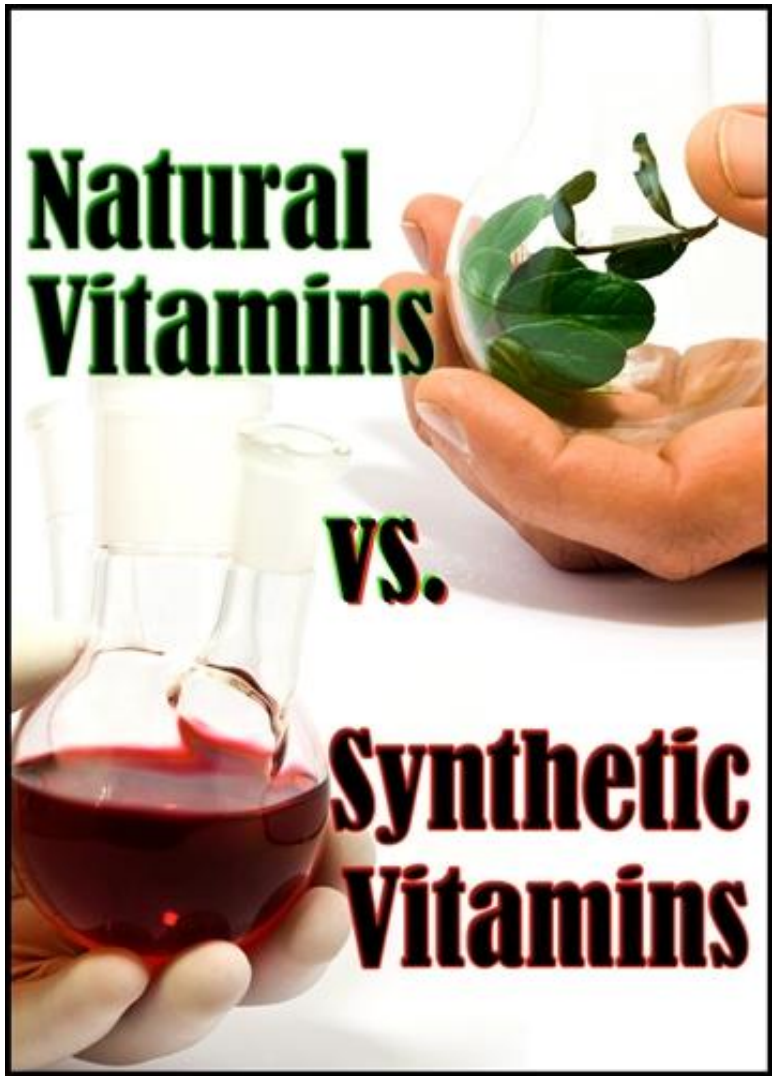

- What is the difference between a “vitamin” that is found in nature and its “synthetic” analog?
- Is there evidence to suggest that supplementing with “natural” vitamins is better or different?

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Photo Credit: Natural Vitamins vs. Synthetic Vitamins – DVD.

“Natural and “Synthetic” - What definition do we use?

- A “natural” vitamin (or mineral) are those which are found (**unmodified**) in nature and are delivered in the food itself, or by concentrating (or isolation) from the natural source of that nutrient
- “Synthetic” is everything else
- Most so-called “synthetics” are bio-identical or bio-equivalent molecules derived from other “naturally” sourced ingredients

Fortified Foods - Confusing the definition of “food-derived”

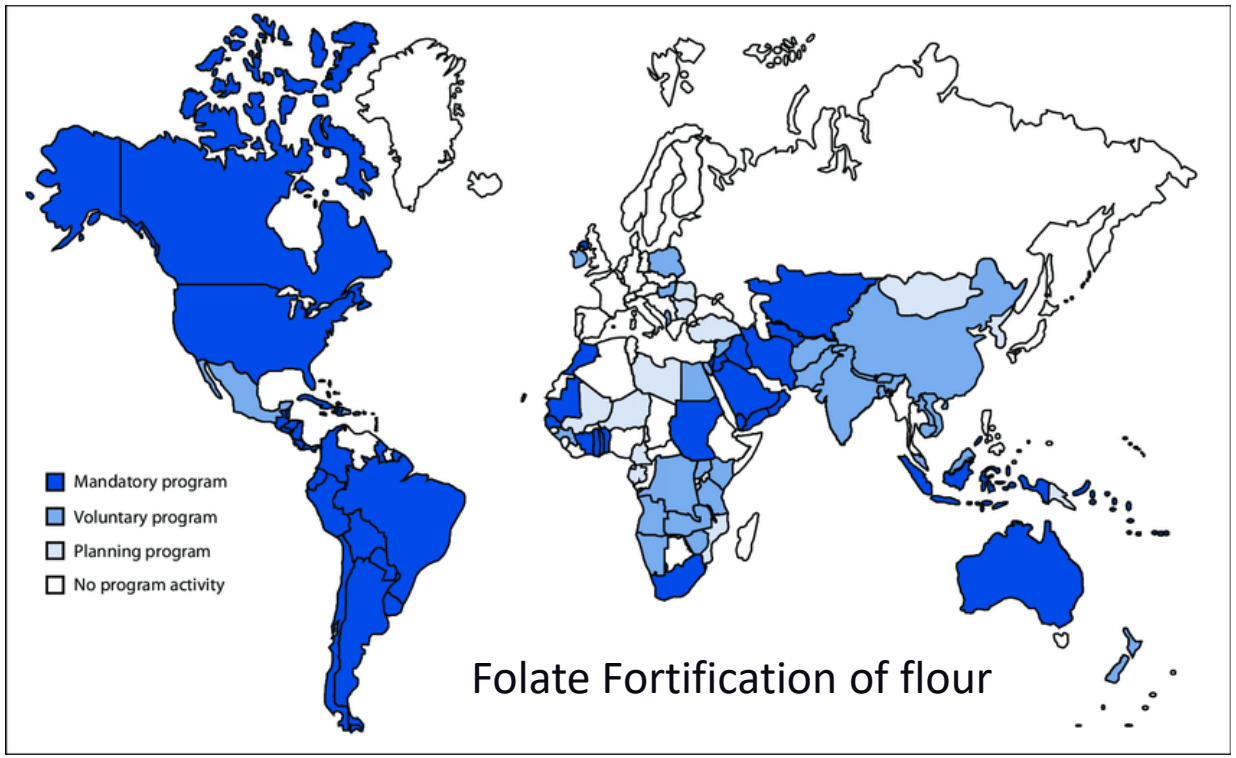


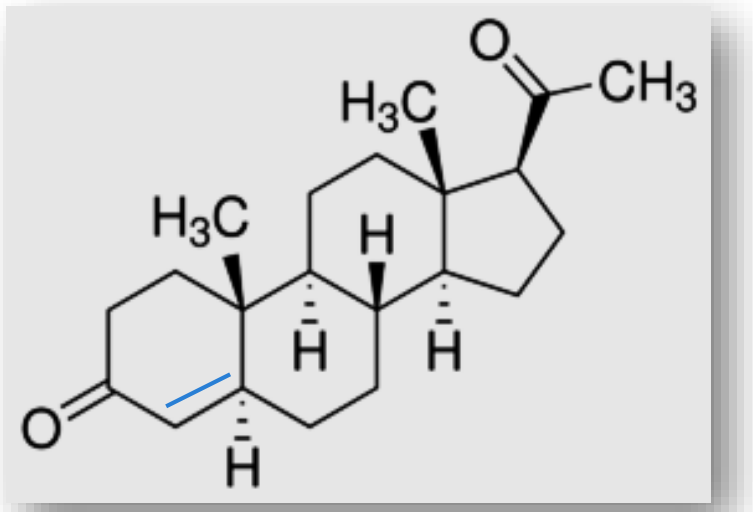
Photo Credit: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5931a2.htm

Structure or Function?

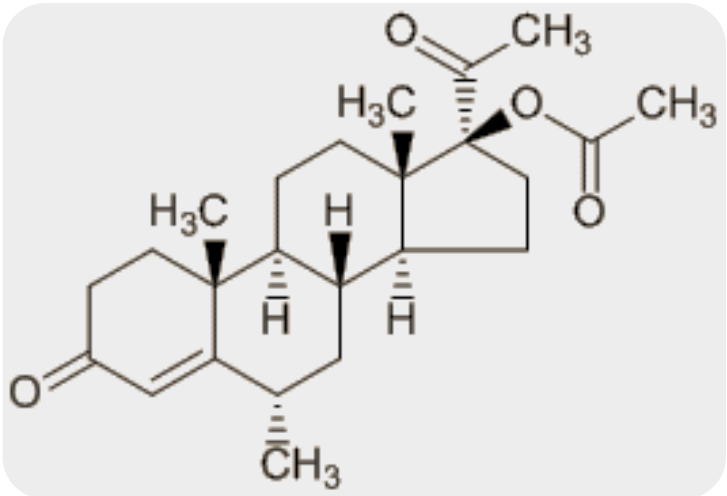
- Bio-Identical: Synthetic compounds whose molecular structure is identical to the compound made in the human (or consumed in unfortified foods)
- Bio-equivalent: Synthetic compounds that can be readily converted by the body into a compound with a function indistinguishable from the compound made in humans or consumed in foods

Bio-identical hormones “Synthetic” or “Natural”

Progesterone



Medroxyprogesterone Acetate



Synthetic Progesterone is neither Bio-identical nor fully Bio-equivalent to the natural progesterone made in the body

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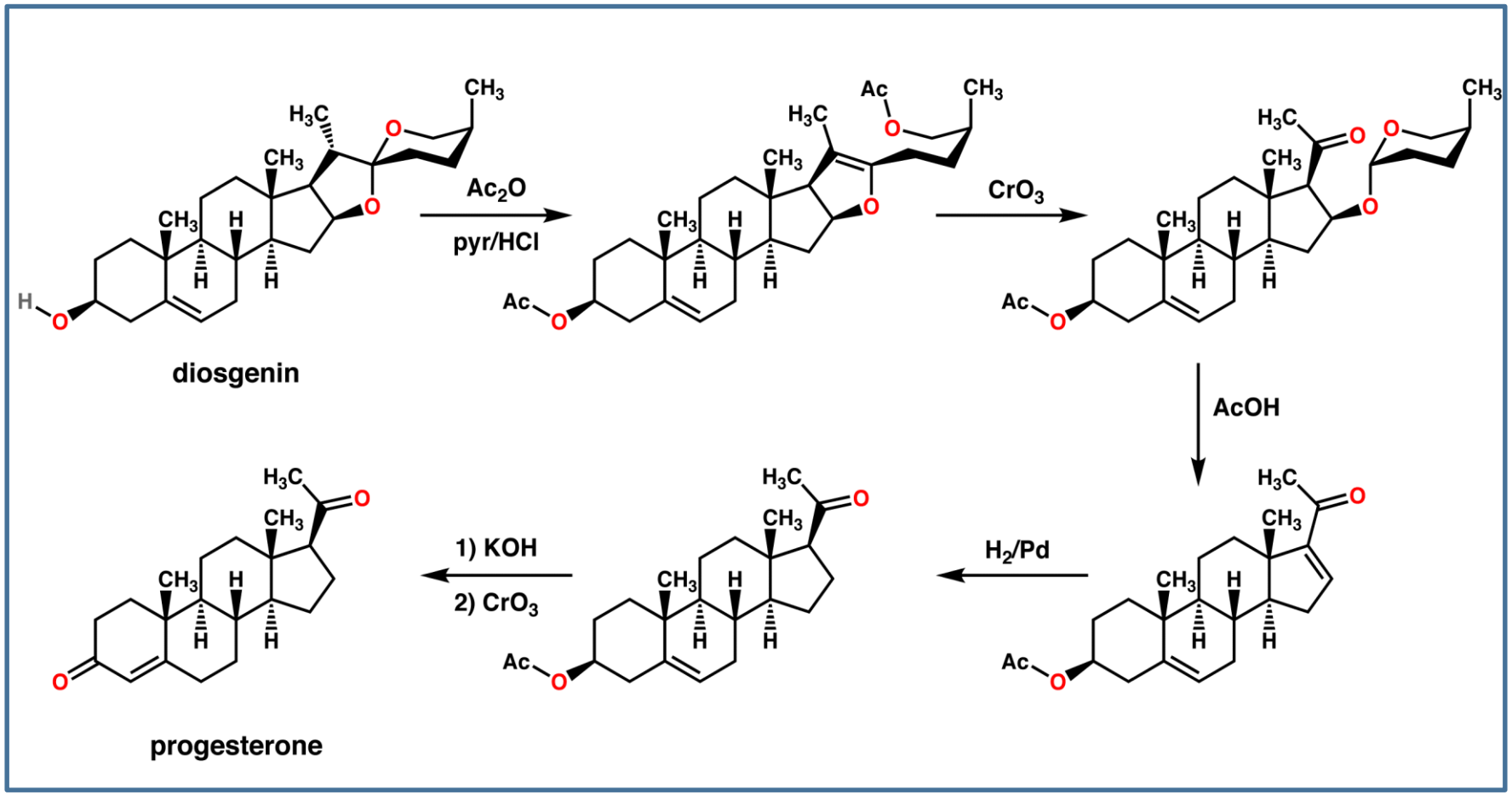
Photo Credit: https://www.researchgate.net/figure/Fig-1-Chemical-structure-of-progesterone_fig1_266385361.

Photo Credit: https://www.tcichemicals.com/eshop/en/in/commodity/M1964/.

Bio-identical hormones “Synthetic” or “Natural”



The Marker semisynthesis of progesterone from diosgenin (Wild Yam)

Photo Credit: https://en.wikipedia.org/wiki/Progesterone.

Naturally Synthesized or Synthesized to be Natural?

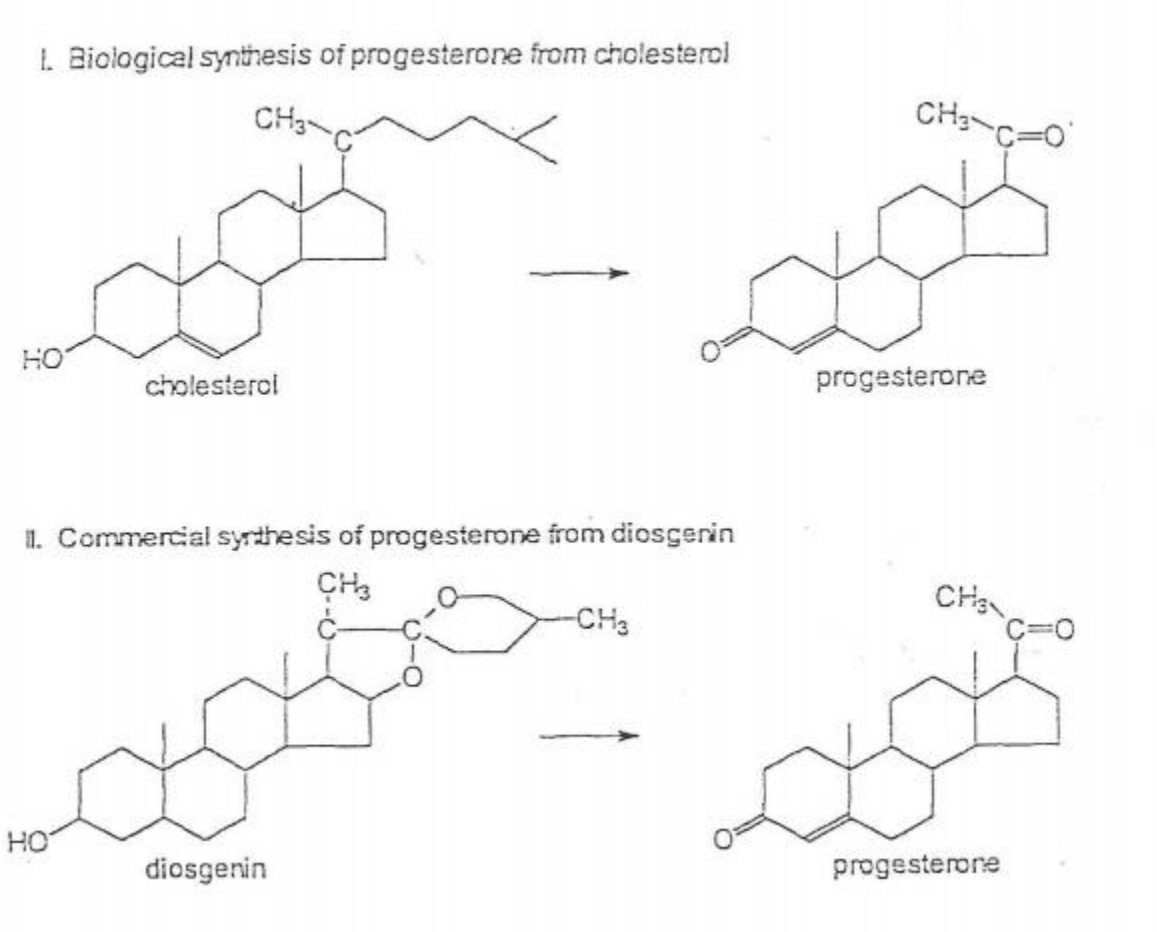


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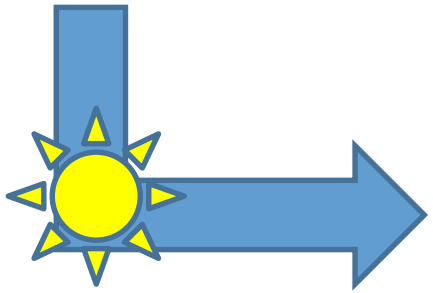
Photo Credit: https://www.i-gap.org/app/dokumente/gynaekologie_kompatibilitaetsmodus.pdf.

How about Vitamin D3?

Sheep Wool Lanolin



Nearly All Supplement Vitamin D3 is derived from UV-exposed Lanolin

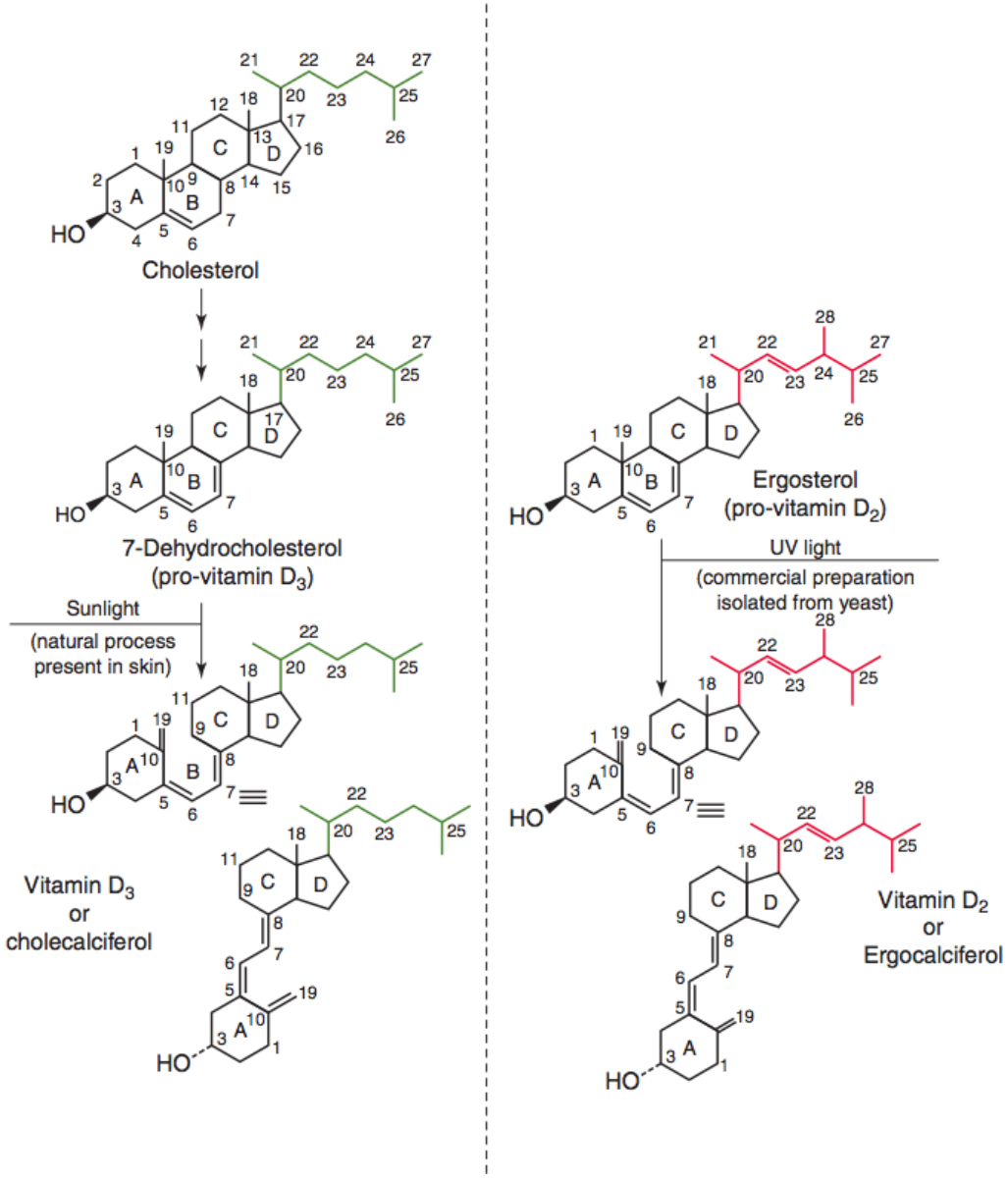
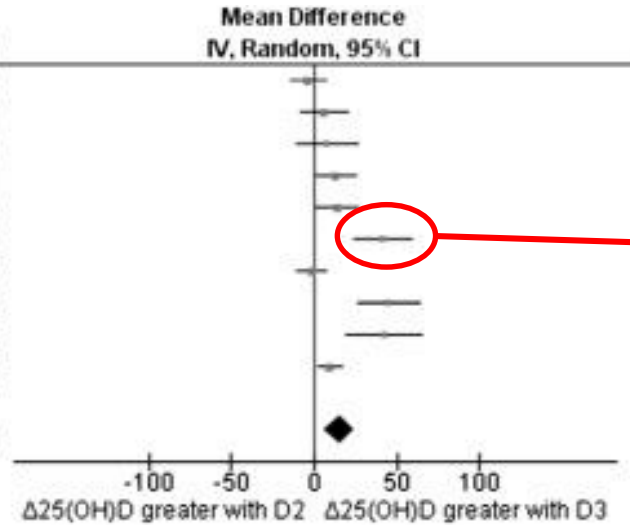


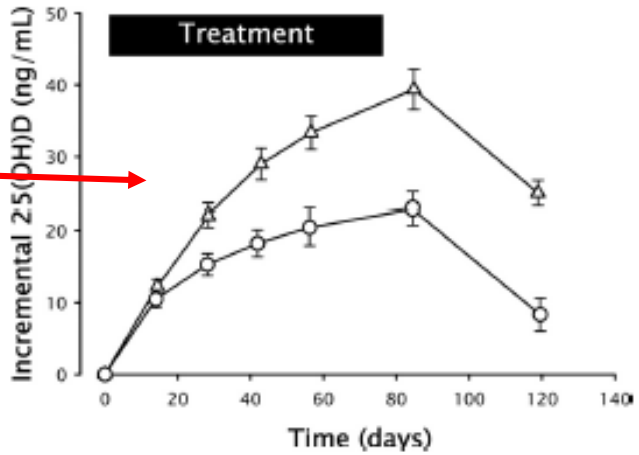
Photo Credit: https://vitamind.ucr.edu/about/

Comparison of vitamin D_2 and vitamin D_3 supplementation in raising serum 25-hydroxyvitamin D status: a systematic review and meta-analysis[1–3]

Study or Subgroup	D3 Mean	D3 SD	D3 Total	D2 Mean	D2 SD	D2 Total	Weight	Mean Difference IV, Random, 95% CI
Biancuzzo 2010-1 (7)	23.3	17.8	20	27	14.8	16	11.2%	-3.70 [-14.35, 6.95]
Biancuzzo 2010-2 (7)	32	25.3	18	26.5	18	17	9.9%	5.50 [-8.99, 19.99]
Binkley 2011-1 (15)	23	33.8	16	15.3	16.5	16	8.6%	7.70 [-10.73, 26.13]
Binkley 2011-2 (15)	22.3	18.3	15	9	14.3	16	10.9%	13.30 [1.69, 24.91]
Glendenning 2009 (16)	40	24.7	17	26	11.2	20	10.5%	14.00 [1.27, 26.73]
Heaney 2011 (17)	98.4	29.1	17	57.4	22	16	8.9%	41.00 [23.46, 58.54]
Holick 2008 (6)	23.3	17.8	20	24.8	8	16	11.8%	-1.50 [-10.23, 7.23]
Romagnoli 2008-1 (5)	70.2	20.8	8	25.5	16.9	8	8.6%	44.70 [26.13, 63.27]
Romagnoli 2008-2 (5)	65.4	30.3	8	23.1	13.8	8	7.2%	42.30 [19.23, 65.37]
Trang 1998 (4)	23.3	15.7	55	13.7	11.4	17	12.3%	9.60 [2.77, 16.43]
Total (95% CI)			**194**			**150**	**100.0%**	**15.23 [6.12, 24.34]**

Heterogeneity: $Tau^2 = 162.74$; $Chi^2 = 47.10$, df = 9 ($P < 0.00001$); $I^2 = 81\%$
Test for overall effect: $Z = 3.28$ ($P = 0.001$)





Overall, the increase in Vitamin D status increases more significantly with oral dosing of D3 compared to D2. This is especially true when using large bolus dosing (typically 50,000 IU once weekly) and less so when comparing smaller daily doses.

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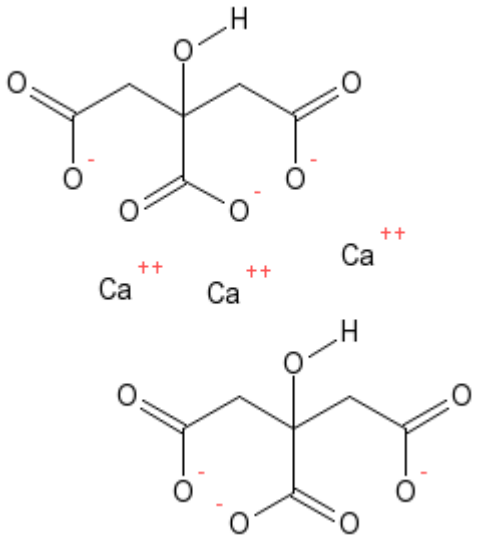
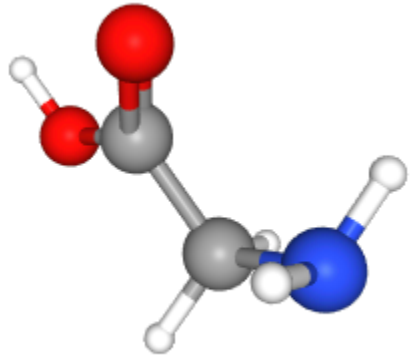
Tripkovic L, et al. *Am J Clin Nutr.* 2012;95(6):1357-64.

Which is more natural?

Calcium Carbonate from Limestone deposits



Calcium Citrate from precipitating citric acid with CaO or CaCarbonate



Calcium bisglycinate Chelate To mimic plant amino acid complexes

Which is a more appropriate supplemental ingredient?

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Photo Credit: https://commons.wikimedia.org/wiki/File:Calcium_citrate.png.

Photo Credit: https://pubchem.ncbi.nlm.nih.gov/compound/Calcium-glycinate

Photo Credit: https://en.wikipedia.org/wiki/Limestone

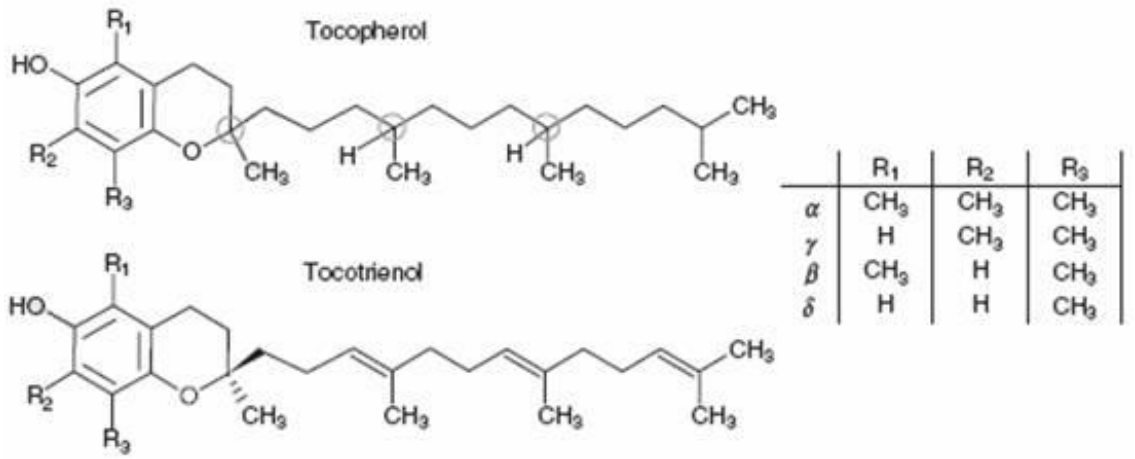
Misinformation and Confusion

- While there are some known differences between some “synthetic” vitamins and their natural counterparts- most of this information is false or greatly exaggerated
- For better or worse- Nearly everything we know about how vitamins work has been accomplished with “synthetic” vitamins
- Research comparing the two is often limited or non-existent

Not all are bio-equivalent

- Synthetic beta-carotene is a single isomer while most food beta-carotene and micro-algae derived concentrates used in supplements are multi-isomers
- Vitamin K1, K2 (multiple forms), and K3 (synthetic)
- Natural Folates, synthetic folates/folic acid
- Synthetic vitamin E isomers vs. natural mixed tocopherols

Vitamin E: Lots of nuance here



	R_1	R_2	R_3
α	CH_3	CH_3	CH_3
γ	H	CH_3	CH_3
β	CH_3	H	CH_3
δ	H	H	CH_3

- Only alpha-tocopherol can be labeled as “Vitamin E”
- While beta, gamma and delta tocopherols are found in foods and have other biological functions, they are not transported by the tocopherol transfer protein and do not substitute for the “vitamin” functions of the alpha form. (Also true for tocotrienols).
- “Mixed-tocopherols” can be listed on the label, but not as “vitamin E”

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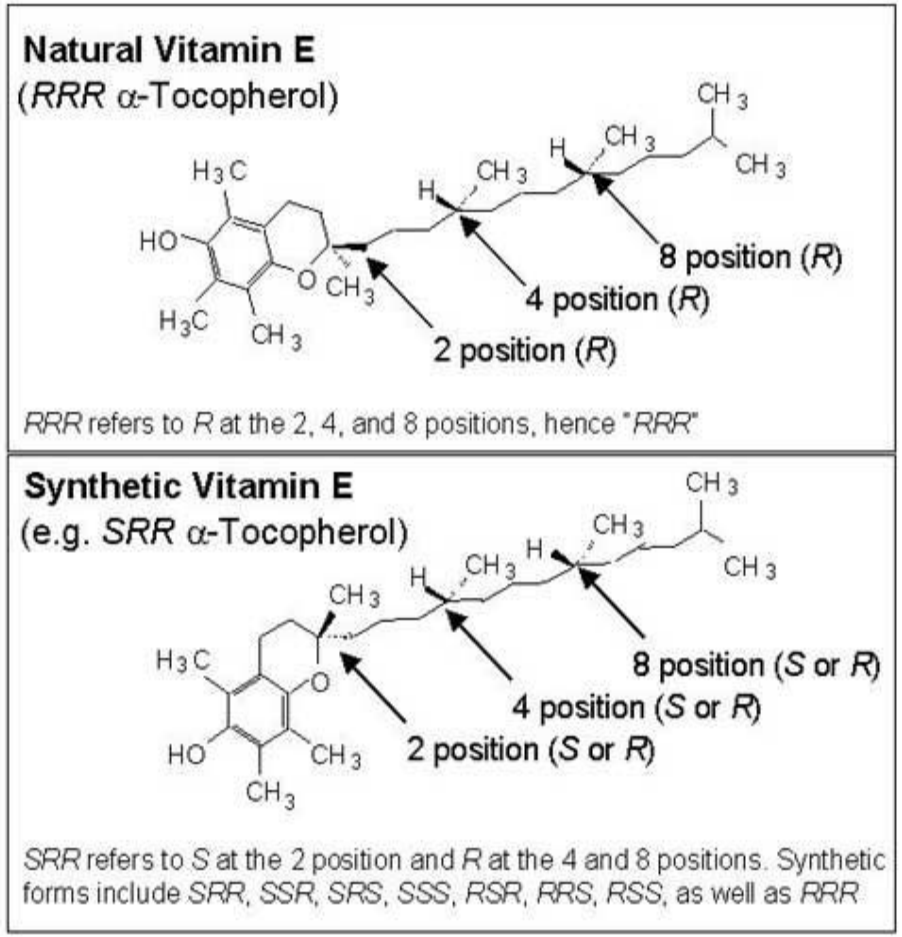
Bartosinska E, et al. *J of Pharm and Biomed Anal.* 2016;127(5):156-169.

Natural vs. Synthetic Vitamin E



- Synthetic E is a blend of 8 racemic isomers.
- "D,L" or all-racemic
- Has lower vitamin E activity compared to natural
- Is adjusted in the "IU" calculation
- Do other isomers interfere?

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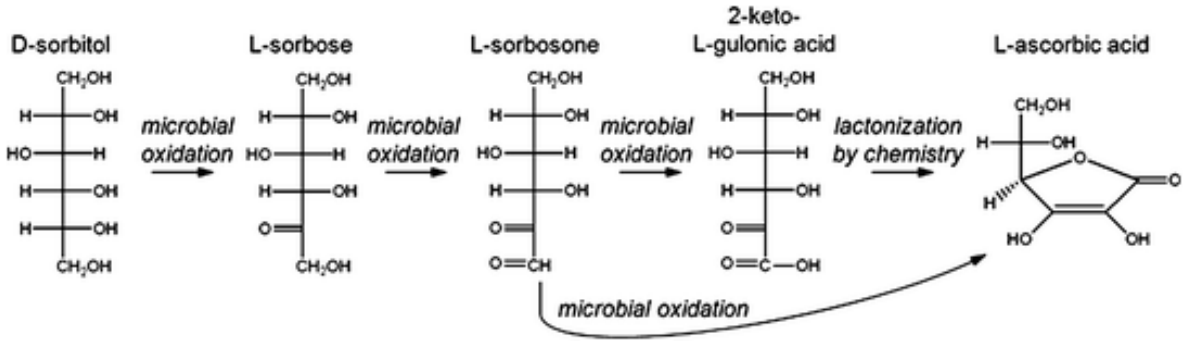
Vagni S, et al. *Food and Nutr Sci.* 2011;2(10):210146.

Vitamin C / Ascorbic Acid



- According to the Linus Pauling Institute:
  - Natural and synthetic L-ascorbic acid are chemically identical, and there are no known differences in their biological activity.
  - A study in 68 male nonsmokers found that ascorbic acid consumed in cooked broccoli, orange juice, orange slices, and as synthetic ascorbic acid tablets are equally bioavailable, as measured by plasma ascorbic acid levels
  - Results from the 10 clinical studies comparing the absorption of vitamin C alone or vitamin C in flavonoid-containing foods showed no appreciable differences in bioavailability of ascorbic acid.

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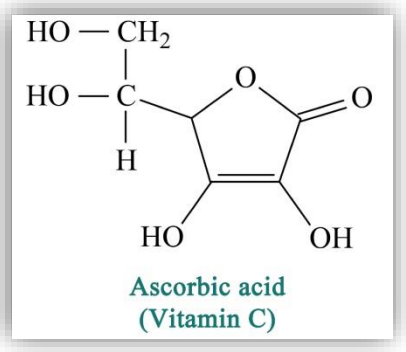
Photo Credit: https://www.studocu.com/en/document/university-of-waikato/process-engineering-and-design/essays/case-study-for-ascorbic-acid/2774154/view

http://lpi.oregonstate.edu leads to a website provided by the Linus Pauling Institute at Oregon State University. Genova Diagnostics is not affiliated or endorsed by the Linus Pauling Institute or Oregon

So Does This Mean?

60 mg of synthetic (ascorbic) Vitamin C = 60 mg of Vitamin C from diet



From the standpoint of Vitamin C activity- Yes

But, from an overall nutrient standpoint- No

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Photo Credit: https://www.researchgate.net/publication/297438049_Determination_of_amount_of_Vitamin_C_Ascorbic_Acid_from_supplied_commercial_tablets_by_using_Iodometric_titration

“Wholefoods Multivitamins”

- This category of products is almost exclusively occupied by products that are not in any way “whole” or “food”
  - Some Products are synthetic vitamins with a blend of fruit/vegetable powders added
  - Many Products are yeast extracts or bran extracts with limited vitamin content
  - Some Products are spiked with synthetics during “fermentation” process and not listed on the label as coming from those compounds
- Products made by combining concentrated food ingredients contain extremely low levels of any vitamins or mineral- most of which are unstable in the manufacturing process

The Irony of Food-Based Nutrients

- Vitamin and mineral content in foods are rarely uniform across different growing and harvesting conditions (very difficult to standardize)
- They are extremely unstable when the food is processed to concentrate the nutrient for use in capsule/tablet/powder. (they degrade quickly)
- FDA GMP-requirements mandate consistent potency and purity throughout the entire shelf-life of the product; making true “whole-food” supplements low potency and much more expensive than the foods they replace

Whole Foods vs Supplements

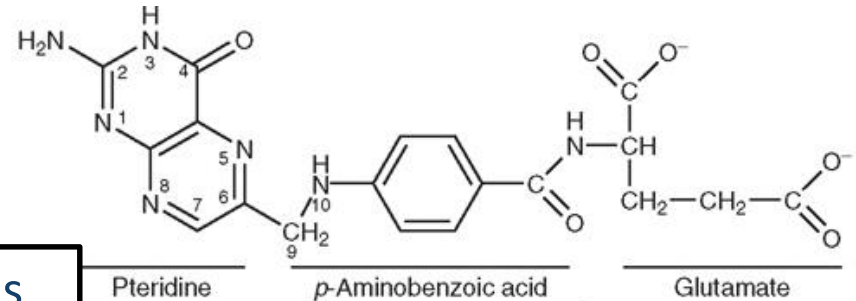
1. In an ideal situation- ALL necessary nutrients would be delivered by a nutrient-dense, unfortified, diverse diet!
2. There are few ideal situations
3. Nutrient Supplementation often requires concentrated isolated nutrients or combinations of these isolated nutrients (often at therapeutic doses)
4. Our body is designed to absorb and store less common nutrients for weeks and even months to ensure our survival- no food contains everything we need in the perfect amount or ratio and no supplemental nutrient (powder or pill) will ever be perfectly suited for every situation
5. Eat the best diet you can, supplement when necessary

Activated B-vitamins: Is there evidence for efficacy?

- Many clinicians have heard (and repeated) that “activated B-vitamins” are needed because the inactive forms don’t work
  - Pyridoxal-5-phosphate (PLP or P5P)
  - Riboflavin-5-phosphate (R5P)
  - 5-Methytetrahydrofolate (5-MTHF)
  - Methylcobalamin (Methyl-B12)
  - Ubiquinol (Reduced form of CoQ10)

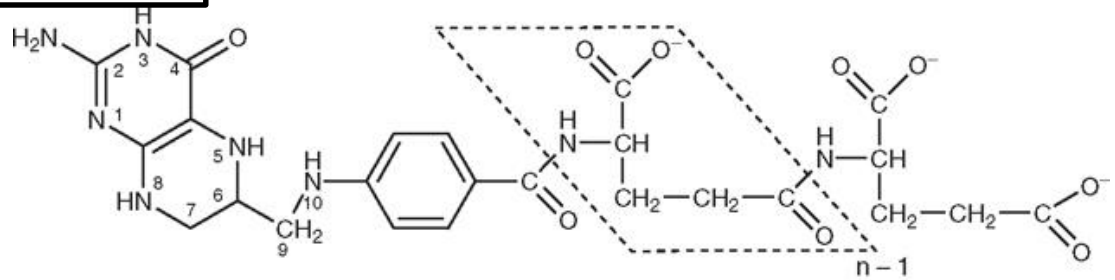
Folate forms: Natural and Synthetic.

Natural folates are polyglutamates



Pteroic acid

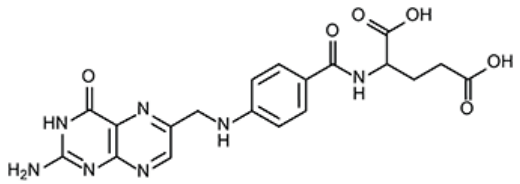
Folic acid, pteroylglutamate (PteGlu)



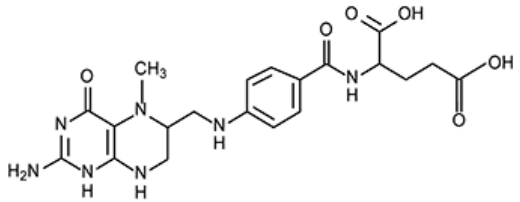
Tetrahydrofolylpoly-γ-glutamate ($H_4PteGlu_n$)

One carbon substituent		Position	Oxidation state
Methyl	$-CH_3$	N-5	Methanol
Methylene	$-CH_2-$	N-5, N-10	Formaldehyde
Methenyl	$-CH=$	N-5, N-10	Formate
Formyl	$-CHO$	N-5 or N-10	Formate
Formimino	$HO=CH-$	N-5	Formate

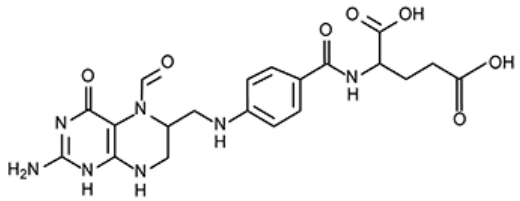
Figure 1. Chemical Structures



Folic acid ($C_{19}H_{19}N_7O_6$)



5-methyltetrahydrofolate ($C_{20}H_{25}N_7O_6$)



Folinic acid ($C_{20}H_{23}N_7O_7$)

All supplemental forms are “synthetic” monoglutamate forms

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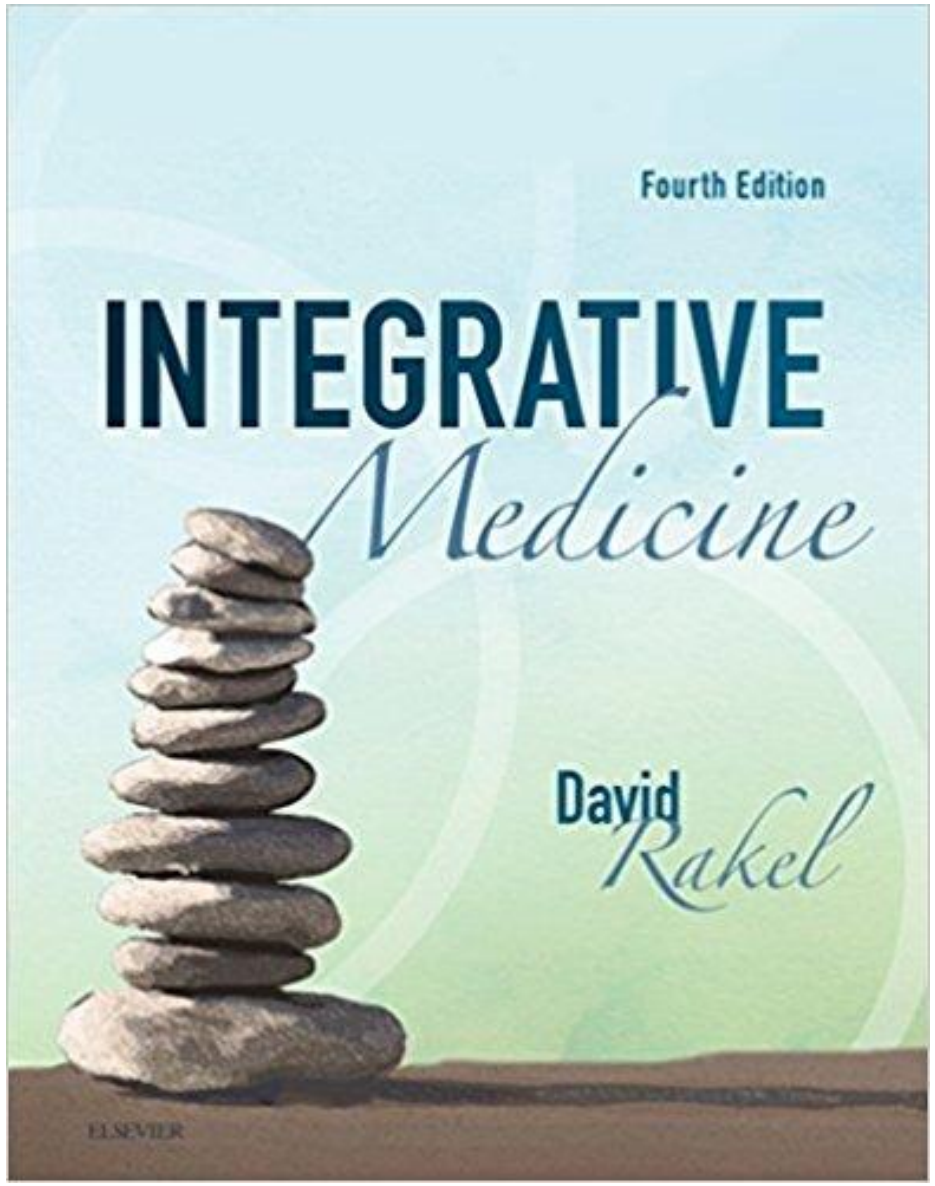
Shane B. *Food Nutr Bull.* 2008;29(Suppl):S5-16.



CHAPTER 38

MTHFR, Homocysteine and Nutrient Needs

Thomas G. Guilliams, PhD

The science of nutrigenetics defines the interrelationship between a person's genetics and their need for, or utilization of, a particular nutrient. As DNA sequencing and nutritional research have become more sophisticated in the past few decades, numerous clinically-relevant nutrigenetic relationships have come to light. Among the most well known are the various polymorphisms in the gene *MTHFR* that encodes the enzyme methylenetetrahydrofolate reductase (MTHFR) needed to synthesize the active form of folate.* In fact, it is likely that *MTHFR* polymorphisms are the most widely-studied of all nutrigenetic-related polymorphisms, the testing for which is now a common clinical practice. This chapter will not attempt to review the vast amount of published data related to the health risks associated with common MTHFR polymorphism (and other mutations).[1] Instead, after describing the biochemistry related to one-carbon metabolism and the role of MTHFR, we will discuss the utility of testing for common polymorphisms to quantify risk in subjects and discuss the available evidence for the use of nutrient supplements to modify a patient's risk based on their *MTHFR* genetics. We will especially focus on the data involving the supplementation of various folates (food folates, folic acid, folinic acid, and 5-MTHF) to reduce risk in patients related to *MTHFR* genetic status, especially as it relates to elevated homocysteine levels (Hcy).

PATHOPHYSIOLOGY

Methylenetetrahydrofolate Reductase—The Folate Activator

MTHFR is a key enzyme in the one-carbon metabolic pathway that regulates methylation via folate and homocysteine metabolism (Fig. 38.1).[2] Specifically, MTHFR catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF). MTHFR is a flavoprotein, which uses flavin adenine dinucleotide (FAD, a riboflavin derivative) as a cofactor and nicotinamide adenine dinucleotide phosphate (NAD[P]H, a niacin derivative) as an electron donor. This reaction is irreversible and provides the only endogenous source of 5-MTHF to the cell. The only known function of 5-MTHF is as a methyl donor in the conversion of homocysteine to methionine, a reaction catalyzed by the enzyme methionine synthase using cobalamin (B12) as a cofactor.[3] Deficiencies in the MTHFR activity lead to reduced cellular availability of 5-MTHF, resulting in elevated serum or urine homocysteine levels.[4] The mechanisms by which MTHFR deficiency influences metabolic dysfunction and disease include the following:

- **Elevated homocysteine.** Elevated plasma total homocysteine (tHcy) is an independent risk factor (or marker) for a wide range of diseases.[5] There are many proposed mechanisms that implicate homocysteine in direct actions on a variety of tissues, although many researchers believe that homocysteine is simply a surrogate biomarker of folate status and/or kidney function and not directly involved in the progression of disease.[6] However, many of the clinical trials in which homocysteine-lowering therapies failed to improve event outcomes were in secondary-prevention populations prescribed a polypharmacy of CVD drugs prior to vitamin consumption (statins, 80%; aspirin, 88%; beta-blockers, 90%; and ACEI, 30%), had baseline tHcy levels below 13 µmol/L, or were unable to achieve posttreatment tHcy levels below 9 µmol/L..[7-9] A wide range of intervention trials have shown that homocysteine-lowering therapies in subjects with mild-to-moderate hyperhomocysteinemia can achieve statistically significant and clinically meaningful benefits.[10-13]

In 358 older adults studied in a community cohort, those with a homocysteine levels >13 µmol/L were almost twice more likely to show decline in memory and global cognitive testing.[10]

- **Increase in the amount or ratio of other folate metabolites.** MTHFR deficiency leads to the likelihood of lower 5-MTHF levels and increasing levels of 5,10-methylenetetrahydrofolate or other nonmethylated forms of folate. While some of these forms are substrates for other metabolic pathways (e.g., for dTMP synthesis; see Fig. 38.1), others may have negative consequences. Some have suggested that elevated 5,10-methylenetetrahydrofolate levels and the subsequent increase in the conversion of dUMP to dTMP may have biological advantages (DNA replication and

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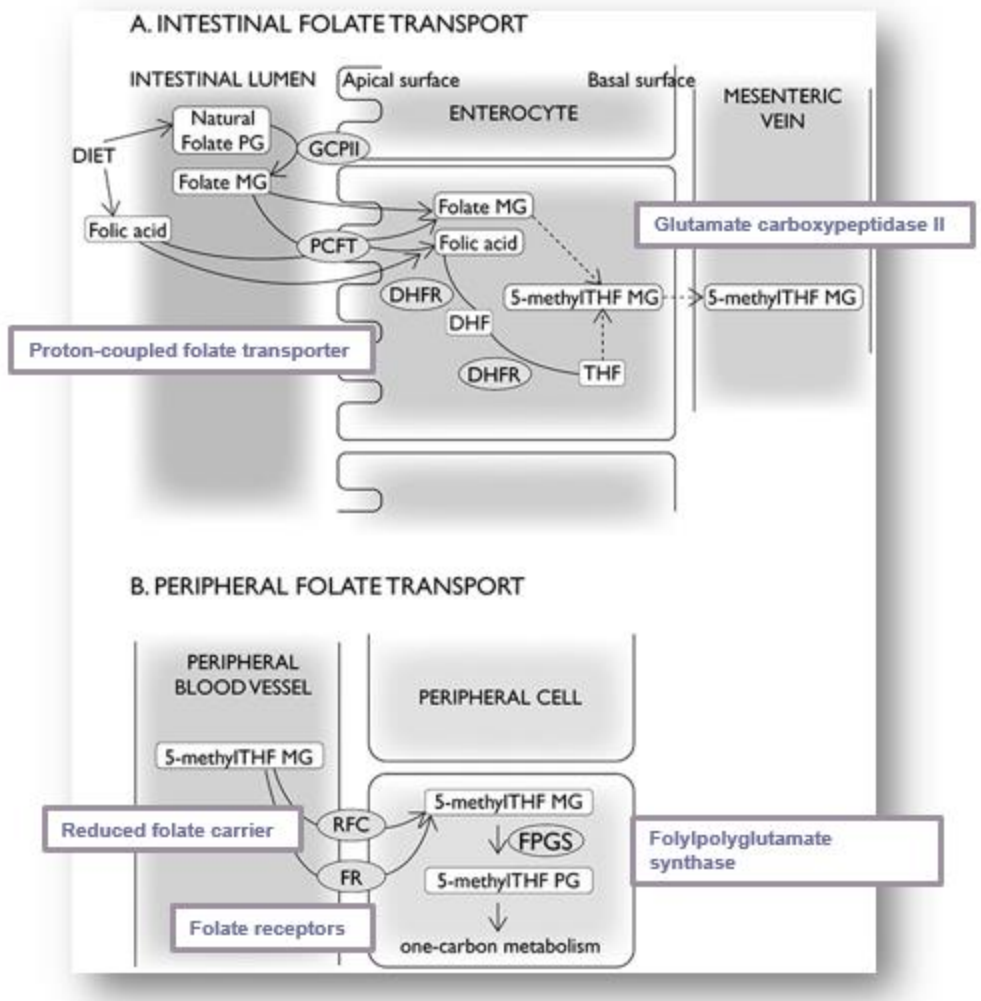
Rakel D. 4th Edition. Saint Louis, Missouri: Elsevier 2017.

Bioavailability of Supplemental vs. Dietary Folates



- Supplemental (folic acid/5mthf) are monoglutamates that absorb at a rate of 1.5-2.5 times higher than polyglutamatic dietary sources
- The new FDA labeling regulations will require each 1 mg of folic acid or 5MTHF to be labeled as 1.7 mg of dietary folate equivalent (DFE)
- Folate transporters only transport monoglutamate forms
- 5MTHF is made into a polyglutamate form in the cell for activity

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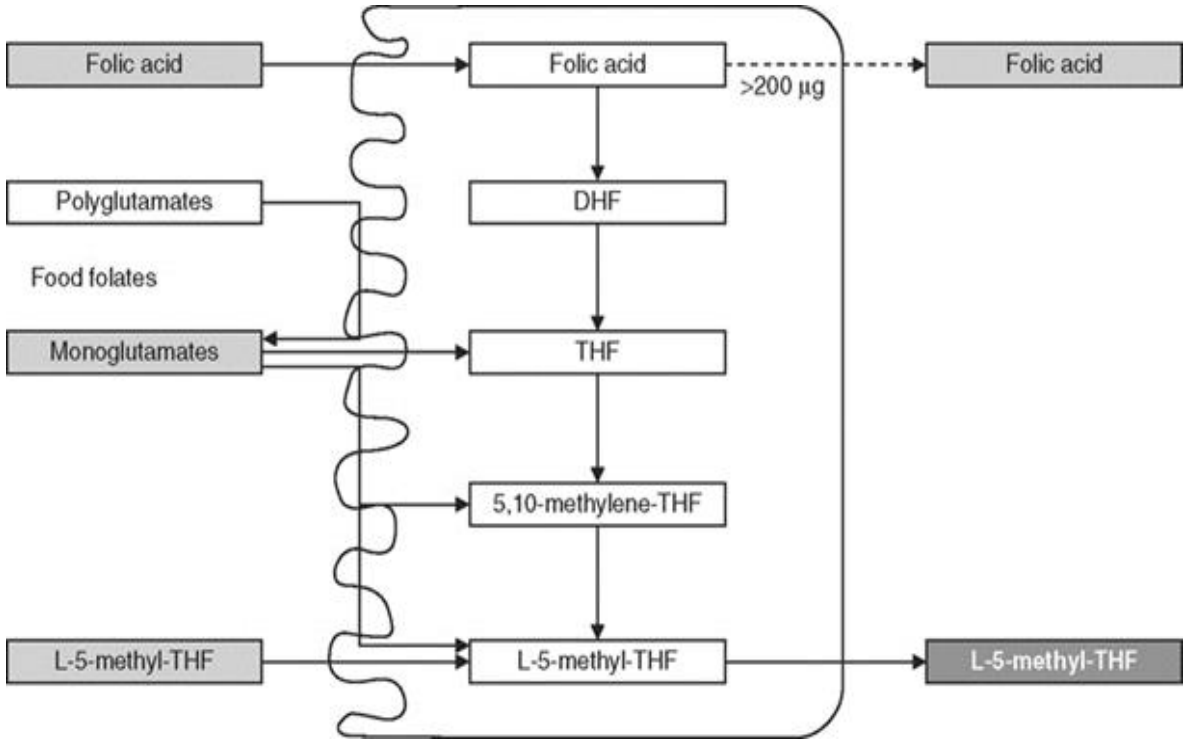
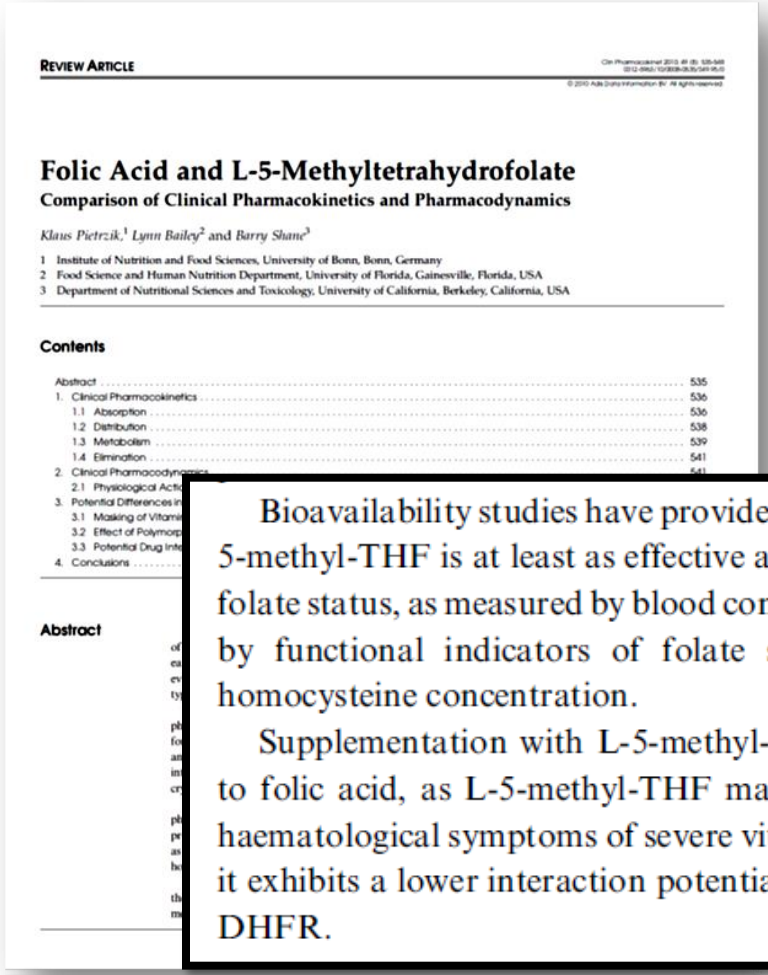
Laanpere M, et al. *Nutr Rev*. 2010;68(2):99-113.

Folic acid vs. 5-MTHF in clinical practice





REVIEW ARTICLE

Folic Acid and L-5-Methyltetrahydrofolate

Comparison of Clinical Pharmacokinetics and Pharmacodynamics

Klaus Pietrzik,[1] Lynn Bailey[2] and Barry Shane[3]

1 Institute of Nutrition and Food Sciences, University of Bonn, Bonn, Germany
2 Food Science and Human Nutrition Department, University of Florida, Gainesville, Florida, USA
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3.1 Masking of Vitami
3.2 Effect of Polymorp
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4. Conclusions .....

Abstract

Bioavailability studies have provided strong evidence that L-5-methyl-THF is at least as effective as folic acid in improving folate status, as measured by blood concentrations of folate and by functional indicators of folate status, such as plasma homocysteine concentration.

Supplementation with L-5-methyl-THF may be preferable to folic acid, as L-5-methyl-THF may be less likely to mask haematological symptoms of severe vitamin B_{12} deficiency and it exhibits a lower interaction potential with drugs that inhibit DHFR.

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Pietrzik K, et al. *Clin Pharmacokinet*. 2010;49(8):535-48.

Bioavailability and Homocysteine-lowering

- In general, 5-MTHF raises folate status and reduces homocysteine more than folic acid; especially in subjects that have C677TT polymorphism
- Head-to-head studies, however, are not always statistically significant for either measure. This is affected by dose
- High doses of folic acid have been associated (in some, but not all studies) with elevated measures of unmetabolized folic acid; with unknown clinical significance

Things to Consider…

- Equimolar amounts of 5-MTHF are up to 200 times more expensive than folic acid (from which it is synthesized)
- The benefits are mostly in persons who are MTHFR C677T homozygous (or heterozygous with poor methylation) or sick individuals where folate methylation may be poor
- 5-MTHF: Preferable in subjects with MTHFR 677TT, or 677CT/1298AC genotypes, or when using folate therapies above 5 mg for any subject
- Folic Acid: Avoid using more than 1 mg in subjects with *MTHFR* 677TT as a precaution against excessive unmetabolized folic acid

Things to Consider…

- Folic acid (or blends) are appropriate for supplementation in (mostly) healthy persons, especially where cost considerations are a factor
- Studies have not confirmed 5MTHF is better than folic acid for NTD in pregnancy- but 5MTHF may be warranted in prenatal supplements where methylation status is unknown (it is too expensive to use for fortification)
- Always remember that most Hcy is bound via disulfide bonds to serum proteins, NAC can help break these bonds and allow for elimination of free Hcy

All Three forms of B6 are bioequivalent

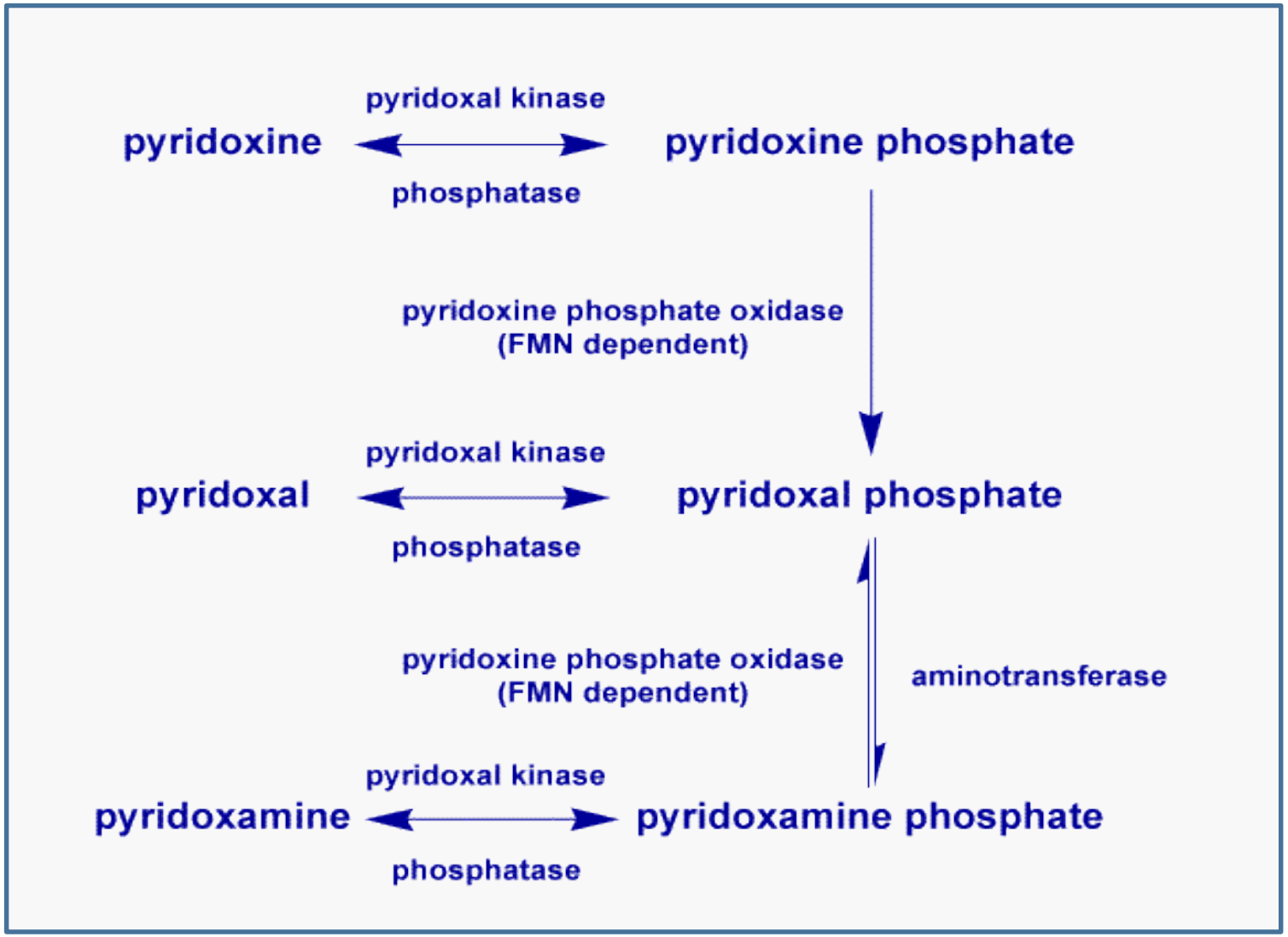

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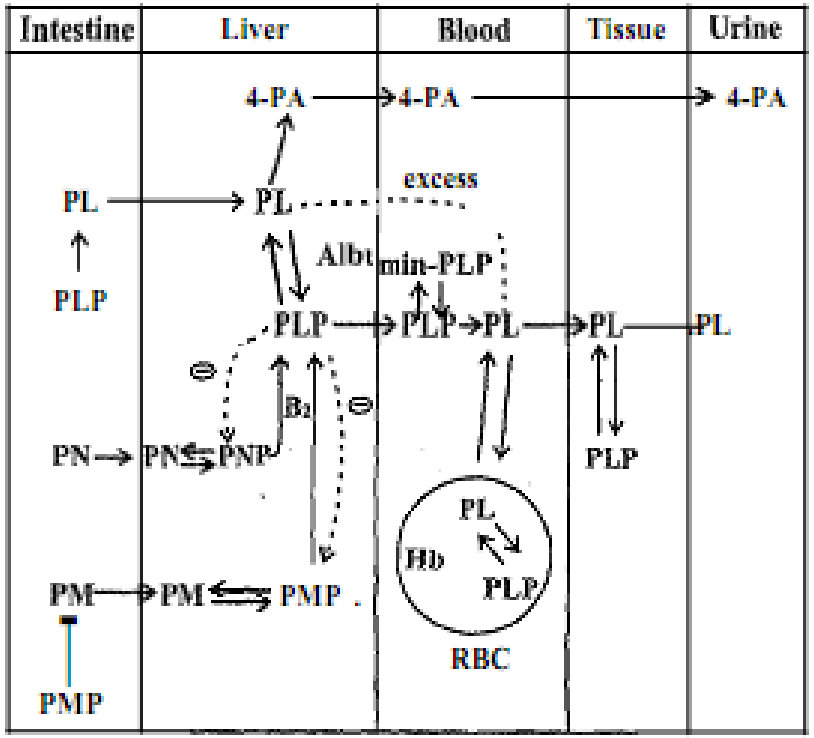
Photo Credit: http://www.siumed.edu/~eniederhoffer/web_lessons/bmb_vit.htm.

Phosphorylated B6 must be dephosphorylated prior to absorption



PL - pyridoxal PN - pyridoxine PM - pyridoxamine 4 PA - pyridoxic acid	PLP - pyridoxal S-phosphate PNP - pyridoxine S-phosphate PMP - pyridoxamine S-phosphate	RBC - red blood cell ⊖ feedback inhibition B_2 - riboflavin

Phosphorylated forms of vitamin B-6 in foods are enzymatically hydrolyzed in the small intestine and absorbed as PN, PL, and PM by a passive diffusion process. Conversion to coenzymic forms takes place in primarily in liver and many other tissues. PLP associated with muscle grycogen phosphorylase is the *in vivo* major pool of vitamin B-6, although this turns over very slowly and undergoes little mobilization to other tissues (Coburn,

P5P is ~ 7-times more expensive than pyridoxine HCl, from which it is synthesized!

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Gregory JF. *Eur J Clin Nutr*. 1997;51 Suppl 1:S43-8.

And Riboflavin?

European Journal of Clinical Nutrition (1997) 51, Suppl. 1, S38-S42

Bioavailability of Riboflavin

CJ Bates

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Descriptors: riboflavin, vitamin B_2, availability, absorption, humans, animal models

Physiology and relevance

Nomenclature

Riboflavin (formerly vitamin G; latterly vitamin B_2) comprises an isoalloxazine ring conjugated to ribitol, the alcohol derived from ribose (Figure 1). In living tissues, the majority is present as either riboflavin phosphate (commonly known as FMN, flavin mononucleotide) or FAD, flavin adenine dinucleotide (Figure 1). Among many analogues of riboflavin, the substitution of ribitol by galactitol to give galactoflavin is perhaps best known as an anti-metabolite (Lambooy, 1975). Of the riboflavin in the body, around 70% is covalently bound to proteins (McCormick, 1989), and like the non-covalently bound forms above, this forms the active site for electron transfer in a wide range of key redox reactions catalysed by flavoprotein enzymes.

Forms in food and dietary sources: RDA

Rich sources of riboflavin in food include offal, yeast hydrolysate, milk, dairy products, and eggs. In contrast, ungerminated grains, especially polished rice, are poor sources, and populations which rely primarily on these are at high risk of deficiency. The non-covalently bound forms in food: principally FMN and FAD, with smaller amounts of free riboflavin, are thought to be entirely and equally available for absorbtion, which involves conversion to free riboflavin by hydrolytic enzymes. The covalently-bound forms such as the cofactor of succinic dehydrogenase, are essentially unavailable and unused. Some riboflavin is synthesized by bacterial metabolism in the colon; however this is poorly available. The US RDAs for riboflavin are: 1.7 mg/d for adult women; 1.3 mg/d for adult men; the equivalent UK Reference Nutrient Intakes (RNI) are: 1.4 mg/d for adult men; 1.2 mg/d for adult women.

Analysis: status indicators

There are basically three categories of analytical procedures that are widely used for food and tissue flavin analysis: (a) microbiological assay, generally using Lactobacillus casei; (b) fluorimetric assay on crude extracts, using a procedure such as chemical reduction or photolytic bleaching to achieve a blank correction, and (c) chromatography such as high pressure liquid chromatography (HPLC) with optical density, fluorescence or electrochemical detection, to achieve high resolution and hence high specificity and sensitivity. Measurement of riboflavin status *in vivo* may employ similar approaches applied to urine or blood, or it may harness a red cell flavoprotein enzyme: glutathione reductase, whose level of saturation with FAD is a good barometer of tissue riboflavin status in animals and man. This status indicator has been extensively used in studies and surveys of human status, usually by means of an 'activation coefficient', or ratio of FAD-reactivated to basal activity, abbreviated to 'EGRAC' from 'erythrocyte glutathione reductase activation coefficient'. Being a ratio of two rates, this index is independent of any uncontrolled denominator, and hence is robust as well as sensitive in practice (Bates, 1987). It fails in a few special situations, however, such as in people with genetic glucose-6-phosphate dehydrogenase deficiency, whose erythrocyte glu-

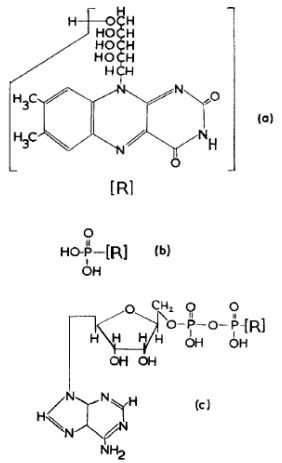


Figure 1 (a) Riboflavin; (b) Riboflavin phosphate (flavin mononucleotide, FMN); (c) Flavin adenine dinucleotide.

Absorption mechanisms

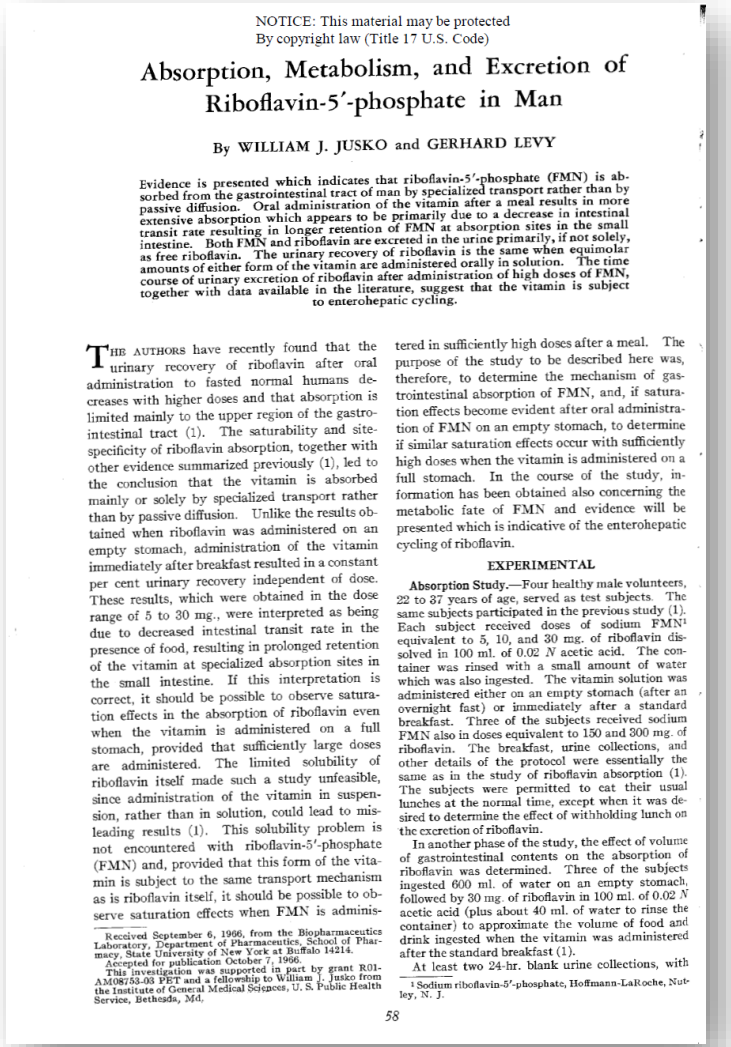
Flavins in foods are converted from the coenzyme forms to free riboflavin by the intestinal digestive enzymes before absorption, and those (covalently bound) forms in foods which cannot be liberated as free riboflavin appear to be unavailable for absorption (McCormick, 1972). However,

Following absorbtion, free riboflavin is phosphorylated within the mucosal cells (which assists transport against the concentration gradient), but the free vitamin in then released again into the bloodstream. There is evidence of

R5P is 3-times more expensive than free riboflavin from which it is synthesized

Bates CJ. *Eur J Clin Nutr*. 1997;51 Suppl 1:S38-42.

A paper as old as me!



Absorption, Metabolism, and Excretion of Riboflavin-5′-phosphate in Man

By WILLIAM J. JUSKO and GERHARD LEVY

Evidence is presented which indicates that riboflavin-5′-phosphate (FMN) is absorbed from the gastrointestinal tract of man by specialized transport rather than by passive diffusion. Oral administration of the vitamin after a meal results in more extensive absorption which appears to be primarily due to a decrease in intestinal transit rate resulting in longer retention of FMN at absorption sites in the small intestine. Both FMN and riboflavin are excreted in the urine primarily, if not solely, as free riboflavin. The urinary recovery of riboflavin is the same when equimolar amounts of either form of the vitamin are administered orally in solution. The time course of urinary excretion of riboflavin after administration of high doses of FMN, together with data available in the literature, suggest that the vitamin is subject to enterohepatic cycling.

THE AUTHORS have recently found that the urinary recovery of riboflavin after oral administration to fasted normal humans decreases with higher doses and that absorption is limited mainly to the upper region of the gastrointestinal tract (1). The saturability and site-specificity of riboflavin absorption, together with other evidence summarized previously (1), led to the conclusion that the vitamin is absorbed mainly or solely by specialized transport rather than by passive diffusion. Unlike the results obtained when riboflavin was administered on an empty stomach, administration of the vitamin immediately after breakfast resulted in a constant per cent urinary recovery independent of dose. These results, which were obtained in the dose range of 5 to 30 mg., were interpreted as being due to decreased intestinal transit rate in the presence of food, resulting in prolonged retention of the vitamin at specialized absorption sites in the small intestine. If this interpretation is correct, it should be possible to observe saturation effects in the absorption of riboflavin even when the vitamin is administered on a full stomach, provided that sufficiently large doses are administered. The limited solubility of riboflavin itself made such a study unfeasible, since administration of the vitamin in suspension, rather than in solution, could lead to misleading results (1). This solubility problem is not encountered with riboflavin-5′-phosphate (FMN) and, provided that this form of the vitamin is subject to the same transport mechanism as is riboflavin itself, it should be possible to observe saturation effects when FMN is administered in sufficiently high doses after a meal. The purpose of the study to be described here was, therefore, to determine the mechanism of gastrointestinal absorption of FMN, and, if saturation effects become evident after oral administration of FMN on an empty stomach, to determine if similar saturation effects occur with sufficiently high doses when the vitamin is administered on a full stomach. In the course of the study, information has been obtained also concerning the metabolic fate of FMN and evidence will be presented which is indicative of the enterohepatic cycling of riboflavin.

EXPERIMENTAL

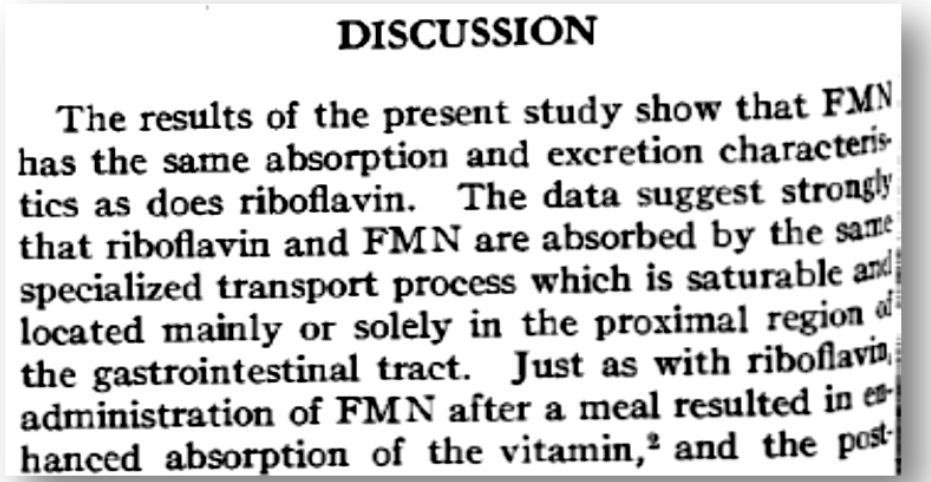
Absorption Study.—Four healthy male volunteers, 22 to 37 years of age, served as test subjects. The same subjects participated in the previous study (1). Each subject received doses of sodium FMN[1] equivalent to 5, 10, and 30 mg. of riboflavin dissolved in 100 ml. of 0.02 *N* acetic acid. The container was rinsed with a small amount of water which was also ingested. The vitamin solution was administered either on an empty stomach (after an overnight fast) or immediately after a standard breakfast. Three of the subjects received sodium FMN also in doses equivalent to 150 and 300 mg. of riboflavin. The breakfast, urine collections, and other details of the protocol were essentially the same as in the study of riboflavin absorption (1). The subjects were permitted to eat their usual lunches at the normal time, except when it was desired to determine the effect of withholding lunch on the excretion of riboflavin.

In another phase of the study, the effect of volume of gastrointestinal contents on the absorption of riboflavin was determined. Three of the subjects ingested 600 ml. of water on an empty stomach, followed by 30 mg. of riboflavin in 100 ml. of 0.02 *N* acetic acid (plus about 40 ml. of water to rinse the container) to approximate the volume of food and drink ingested when the vitamin was administered after the standard breakfast (1).

At least two 24-hr. blank urine collections, with

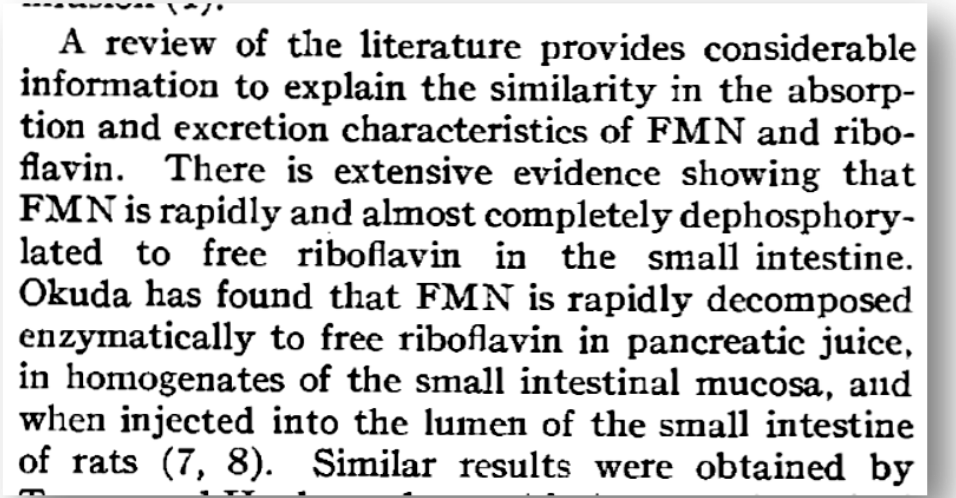
Received September 6, 1966, from the Biopharmaceutics Laboratory, Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo 14214.
Accepted for publication October 7, 1966.
This investigation was supported in part by grant R01-AM08753-03 PET and a fellowship to William J. Jusko from the Institute of General Medical Sciences, U. S. Public Health Service, Bethesda, Md.

[1] Sodium riboflavin-5′-phosphate, Hoffmann-LaRoche, Nutley, N. J.



DISCUSSION

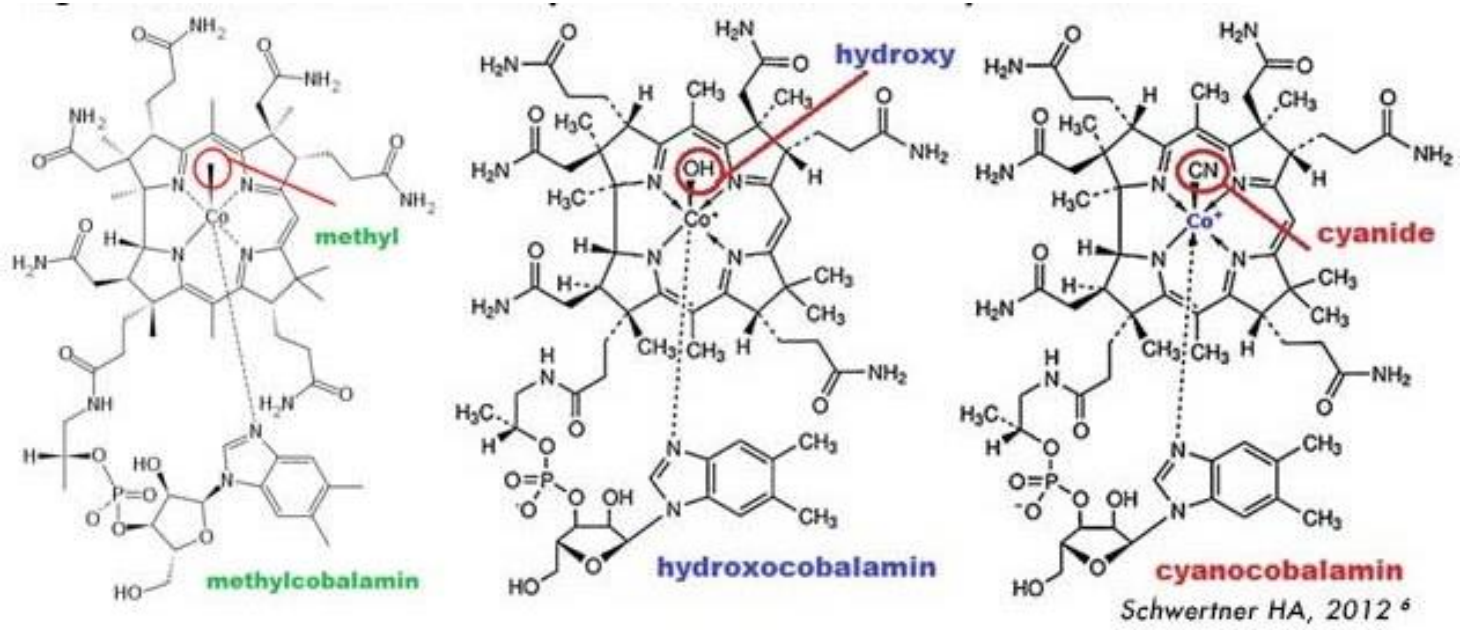
The results of the present study show that FMN has the same absorption and excretion characteristics as does riboflavin. The data suggest strongly that riboflavin and FMN are absorbed by the same specialized transport process which is saturable and located mainly or solely in the proximal region of the gastrointestinal tract. Just as with riboflavin, administration of FMN after a meal resulted in enhanced absorption of the vitamin,[2] and the post-



A review of the literature provides considerable information to explain the similarity in the absorption and excretion characteristics of FMN and riboflavin. There is extensive evidence showing that FMN is rapidly and almost completely dephosphorylated to free riboflavin in the small intestine. Okuda has found that FMN is rapidly decomposed enzymatically to free riboflavin in pancreatic juice, in homogenates of the small intestinal mucosa, and when injected into the lumen of the small intestine of rats (7, 8). Similar results were obtained by

GENOVA DIAGNOSTICS

Jusko WJ, et al. *J Pharm Sci*. 1967;56(1):58-62.



- Comparisons between different forms of cobalamin (B12) are virtually non-existent
- Nearly all studies done (until recently) have been performed with cyanocobalamin
- MethylB12 is only about 1.5 to 3 times more expensive- and rarely impacts cost of finished product
- Since methylB12 is more common in nature- it is favorable over cyanoB12, or hydroxyB12 for use as a supplement; although no credible data tells us that cyanoB12 is either unsafe or ineffective!

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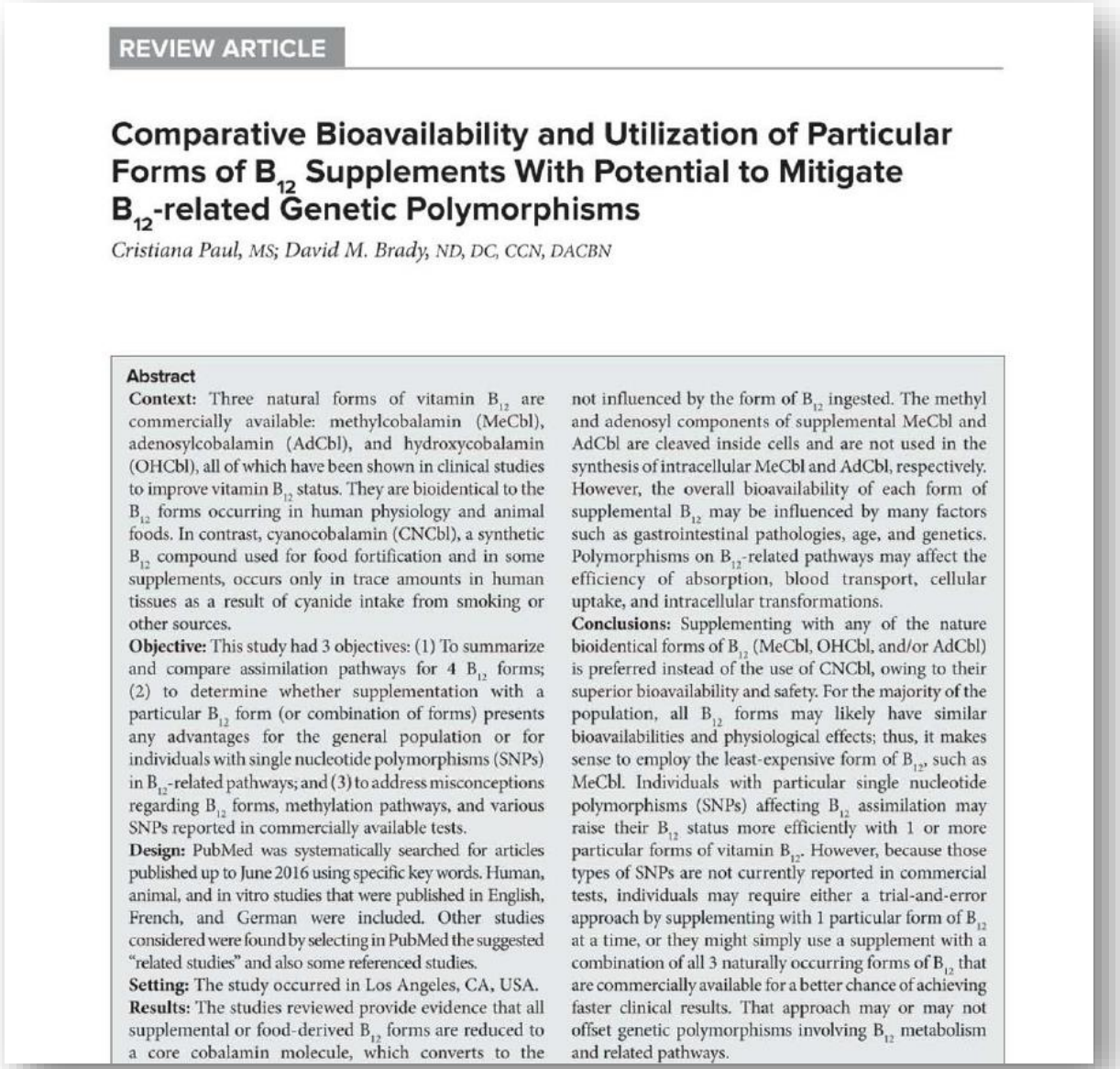
Photo Credit: Schwertner H, 2012.



REVIEW ARTICLE

Comparative Bioavailability and Utilization of Particular Forms of B_{12} Supplements With Potential to Mitigate B_{12}-related Genetic Polymorphisms

Cristiana Paul, MS; David M. Brady, ND, DC, CCN, DACBN

Abstract

Context: Three natural forms of vitamin B_{12} are commercially available: methylcobalamin (MeCbl), adenosylcobalamin (AdCbl), and hydroxycobalamin (OHCbl), all of which have been shown in clinical studies to improve vitamin B_{12} status. They are bioidentical to the B_{12} forms occurring in human physiology and animal foods. In contrast, cyanocobalamin (CNCbl), a synthetic B_{12} compound used for food fortification and in some supplements, occurs only in trace amounts in human tissues as a result of cyanide intake from smoking or other sources.

Objective: This study had 3 objectives: (1) To summarize and compare assimilation pathways for 4 B_{12} forms; (2) to determine whether supplementation with a particular B_{12} form (or combination of forms) presents any advantages for the general population or for individuals with single nucleotide polymorphisms (SNPs) in B_{12}-related pathways; and (3) to address misconceptions regarding B_{12} forms, methylation pathways, and various SNPs reported in commercially available tests.

Design: PubMed was systematically searched for articles published up to June 2016 using specific key words. Human, animal, and in vitro studies that were published in English, French, and German were included. Other studies considered were found by selecting in PubMed the suggested "related studies" and also some referenced studies.

Setting: The study occurred in Los Angeles, CA, USA.

Results: The studies reviewed provide evidence that all supplemental or food-derived B_{12} forms are reduced to a core cobalamin molecule, which converts to the not influenced by the form of B_{12} ingested. The methyl and adenosyl components of supplemental MeCbl and AdCbl are cleaved inside cells and are not used in the synthesis of intracellular MeCbl and AdCbl, respectively. However, the overall bioavailability of each form of supplemental B_{12} may be influenced by many factors such as gastrointestinal pathologies, age, and genetics. Polymorphisms on B_{12}-related pathways may affect the efficiency of absorption, blood transport, cellular uptake, and intracellular transformations.

Conclusions: Supplementing with any of the nature bioidentical forms of B_{12} (MeCbl, OHCbl, and/or AdCbl) is preferred instead of the use of CNCbl, owing to their superior bioavailability and safety. For the majority of the population, all B_{12} forms may likely have similar bioavailabilities and physiological effects; thus, it makes sense to employ the least-expensive form of B_{12}, such as MeCbl. Individuals with particular single nucleotide polymorphisms (SNPs) affecting B_{12} assimilation may raise their B_{12} status more efficiently with 1 or more particular forms of vitamin B_{12}. However, because those types of SNPs are not currently reported in commercial tests, individuals may require either a trial-and-error approach by supplementing with 1 particular form of B_{12} at a time, or they might simply use a supplement with a combination of all 3 naturally occurring forms of B_{12} that are commercially available for a better chance of achieving faster clinical results. That approach may or may not offset genetic polymorphisms involving B_{12} metabolism and related pathways.

- Excellent recent summary of the evidence comparing all forms of vitamin B12
- Virtually no data suggesting any form has any particular advantage over any other

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Paul C, et al. *Integr Med (Encinitas).* 2017;16(1):42-49.

Methyl-B12 Does not Speed up Methylation!

1364 DOI 10.1002/mnfr.201500019 *Mol. Nutr. Food Res.* 2015, *59*, 1364–1372

REVIEW

Cobalamin coenzyme forms are not likely to be superior to cyano- and hydroxyl-cobalamin in prevention or treatment of cobalamin deficiency

Rima Obeid[1,2], Sergey N. Fedosov[3,4] and Ebba Nexo[3]

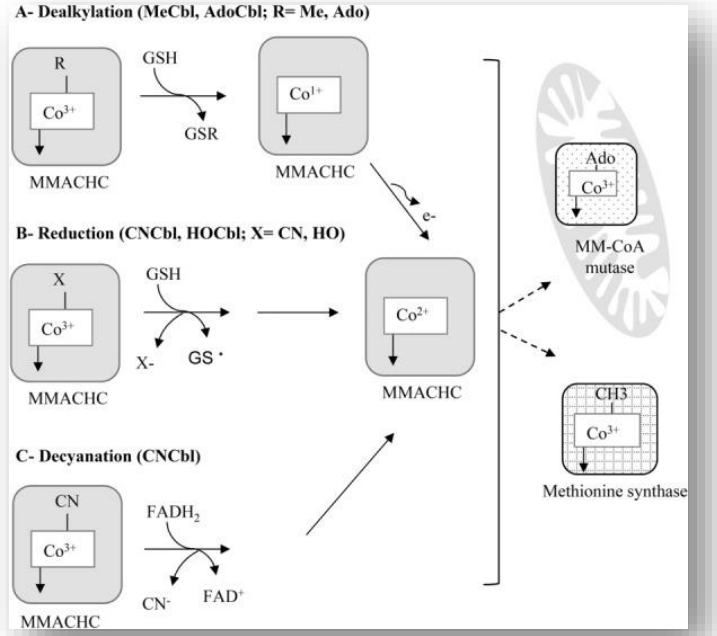
[1] Aarhus Institute of Advanced Studies, University of Aarhus, Aarhus, Denmark
[2] Department of Clinical Chemistry, University Hospital of the Saarland, Homburg, Germany
[3] Department of Clinical Biochemistry, Aarhus University Hospital, Aarhus C, Denmark
[4] Department of Molecular Biology and Genetics, Aarhus University, Aarhus C, Denmark

Received: January 8, 2015
Revised: March 25, 2015
Accepted: March 25, 2015

Methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) are coenzymes for methionine synthase and methylmalonyl-CoA mutase, respectively. Hydroxylcobalamin (HOCbl) and cyanocobalamin (CNCbl) are frequently used for supplementation. MeCbl and AdoCbl have recently emerged as alternative forms in supplements. In the light of metabolic transformation of Cbl into its cofactor forms, this review discusses current evidence on efficacy and utility of different Cbl forms in preventing or treating Cbl deficiency. Cbl-transporting proteins bind and mediate the uptake of all aforementioned forms of Cbl. After internalization and lysosomal release, Cbl binds to the cytosolic chaperon MMACHC that is responsible for (i) flavin-dependent decyanation of [CN-Co^{3+}]Cbl to [Co^{2+}]Cbl; (ii) glutathione-dependent dealkylation of MeCbl and AdoCbl to [$Co^{2+/1+}$]Cbl; and (iii) glutathione-dependent decyanation of CNCbl or reduction of HOCbl under anaerobic conditions. MMACHC shows a broad specificity for Cbl forms and supplies the Cbl^{2+} intermediate for synthesis of MeCbl and AdoCbl. Cobalamin chemistry, physiology, and biochemistry suggest that MeCbl and AdoCbl follow the same route of intracellular processing as CNCbl does. We conclude that supplementing MeCbl or AdoCbl is unlikely to be advantageous compared to CNCbl. On the other hand, there are obvious advantages of high parenteral doses (1–2 mg) of HOCbl in treating inborn errors of Cbl metabolism.

Keywords:
Adenosylcobalamin / Coenzyme / Cyanocobalamin / Deficiency / Hydroxylcobalamin / Methylcobalamin

Additional supporting information may be found in the online version of this article at the publisher's web-site



Methylmalonic aciduria and homocystinuria type C protein removes the methyl from methyl-B12 just as it does the CN, OH, or Adenosyl groups!

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Obeid R, et al. *Mol Nutr Food Res.* 2015;59(7):1364-72.

Injectable or Sublingual B12

- Sublingual B12 is not legally a dietary supplement and is unnecessary anyway
- Oral (swallowed) B12 at 1 mg raises serum levels similarly to intramuscular injections
- At this dose, passive absorption appears to be used and intrinsic factor is less critical

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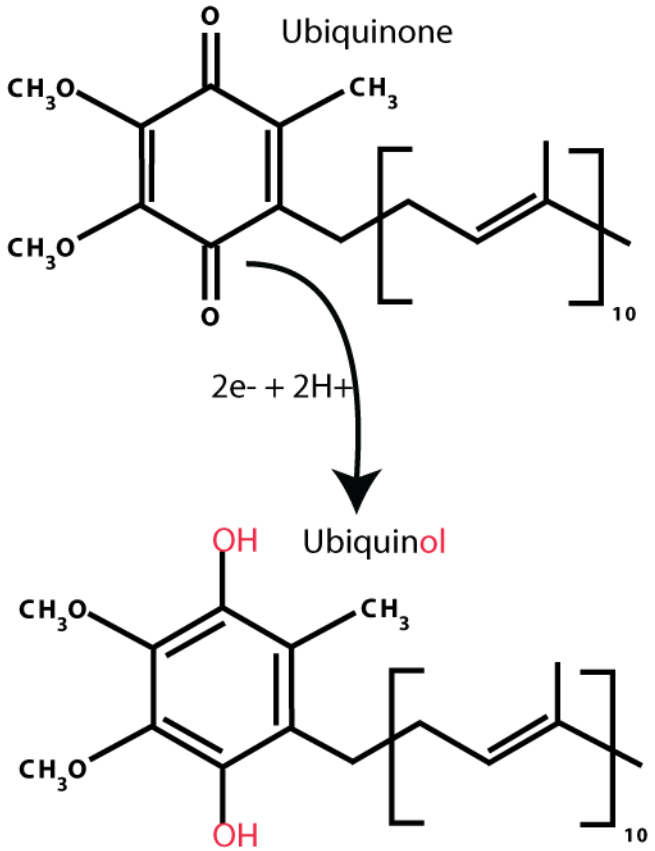
Vidal-Alaball J, et al. *Cochrane Database Syst Rev.* 2005;(3):CD004655.

CoQ10-Ubiquinol



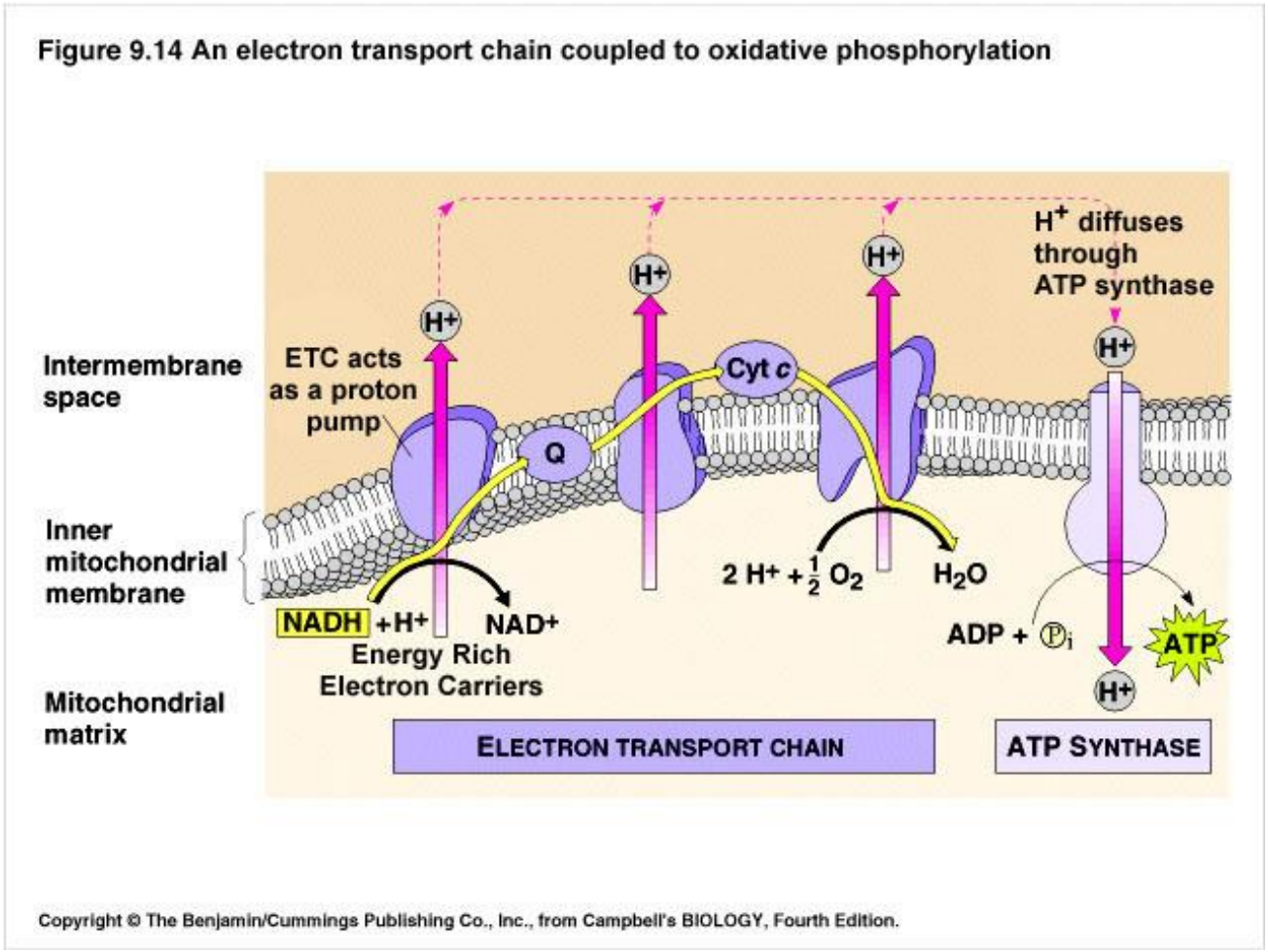


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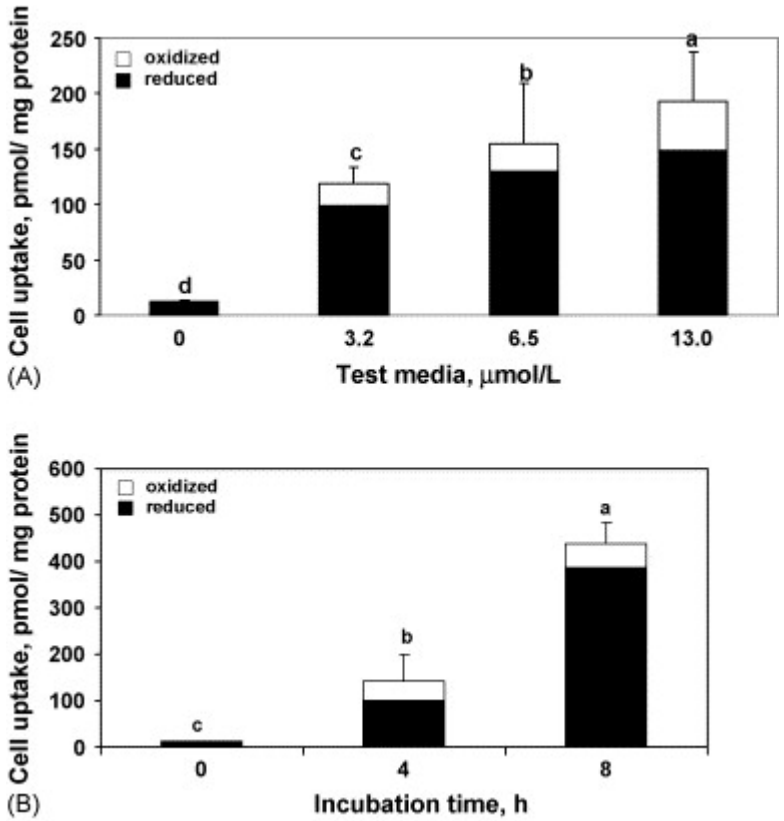
Photo Credit: Copyright Benjamin/Cummings Publishing Co & Triples in LA

Cellular Uptake and Conversion of Ubiquinone (oxidized)

"That the pharmacokinetic profiles of ubiquinone and ubiquinol are identical is not surprising due to the fact that circulating CoQ10 is almost entirely in the form of ubiquinol and that the conversion of ubiquinone to ubiquinol occurs in the enterocytes prior to its lymphatic transport into circulation."



GENOVA DIAGNOSTICS

Bhagavan HN, et al. *Int J Pharm.* 2007;333(1-20'112-17.

Activated Forms: Bottom line

- All vitamins function in the body by a constant conversion between various active and inactive forms
  - In many cases, active vitamins are converted to their inactive forms (intentionally) by specific enzymes in order to transport vitamins from one part of the body to another
  - Inactivating the vitamin prevents it from reacting prior to reaching the target tissue
- Even in the case of methylated folate, the amount of stored body folate that needs to be continually re-methylated could never be overcome by oral methylfolate supplementation alone

Fish Oil: the Products

- Unconcentrated Fish Body Oil: natural triglycerides (TG) containing ~30% EPA/DHA (ratio species and water temp dependent)
- Concentrated Oil:
  - Free Fatty Acids (FFA): concentrated EPA,DHA fatty acids cleaved from TG backbone
  - Ethyl Esters (EE): FFA stabilized with ethanol to create an ester
  - reEsterified TG (rTG): EE re-attached to glycerol backbone to form a bio-identical natural TG with higher % EPA and DHA

Other Sources of EPA/DHA

- Cod Liver: Unconcentrated TG oil similar to Fish Body oil
- Krill: Crustacean with very low EPA/DHA, contains astaxanthin (PL and FFA forms)
- Calamari: Unconcentrated TG, slightly higher DHA
- Mussels: Not typically used to deliver EPA/DHA content
- Algae: high in DHA (TG form)
- GMO plants: Used for feeding farmed fish, not yet approved for direct human consumption

The Molecules

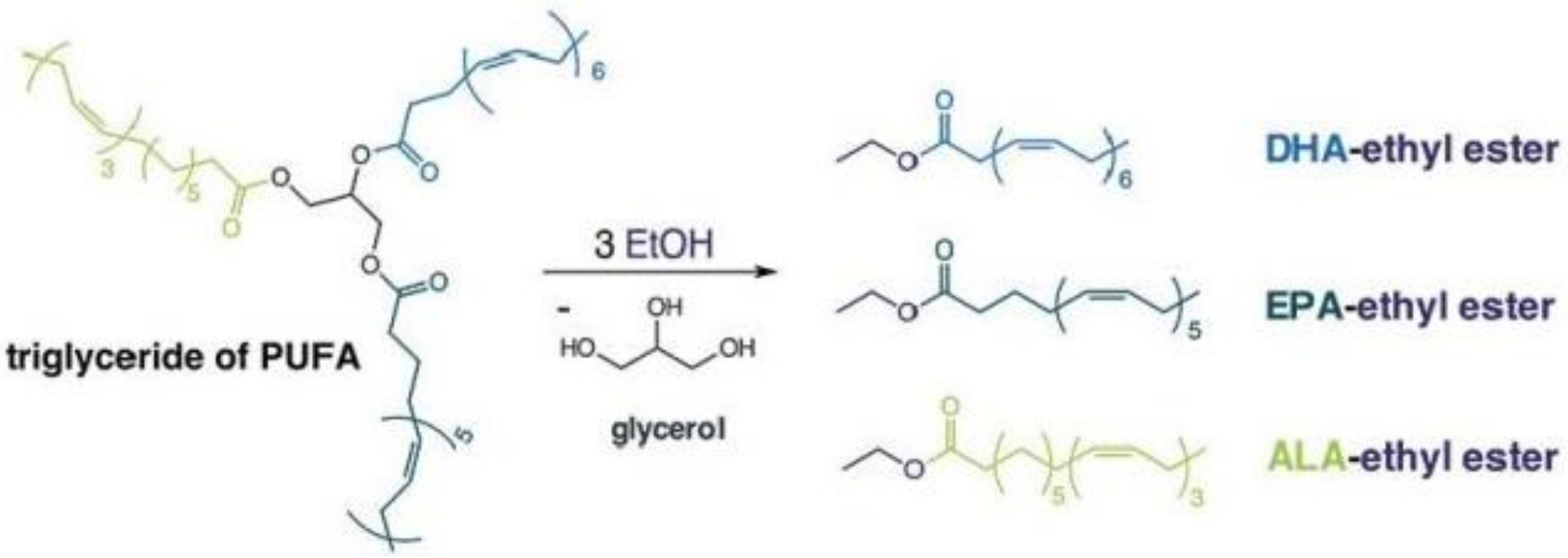


Photo Credit: Examining the Constituents of Fish Oil Supplements Using the NMReady-60e Benchtop.

Disputes over Bioavailability and Clinical Efficacy

- Several Small studies showed no bioavailability difference between rTG and EE
- Several Small studies showed rTG had significantly more bioavailability
- Several studies showed that EE bioavailability is compromised away from fatty meals
- No study shows EE to have better bioavailability over TG

Prostaglandins, Leukotrienes and Essential Fatty Acids 83 (2010) 137–141

Contents lists available at ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Bioavailability of marine n-3 fatty acid formulations☆

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Stereoisomery

ABSTRACT

The use of marine n-3 polyunsaturated fatty acids (n-3 PUFA) as supplements has prompted the development of concentrated formulations to overcome compliance problems. The present study compares three concentrated preparations — ethyl esters, free fatty acids and re-esterified triglycerides — with placebo oil in a double-blinded design, and with fish body oil and cod liver oil in single-blinded arms. Seventy-two volunteers were given approximately 3.3 g of eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) daily for 2 weeks. Increases in absolute amounts of EPA and DHA in fasting serum triglycerides, cholesterol esters and phospholipids were examined. Bioavailability of EPA+DHA from re-esterified triglycerides was superior (124%) compared with natural fish oil, whereas the bioavailability from ethyl esters was inferior (73%). Free fatty acid bioavailability (91%) did not differ significantly from natural triglycerides. The stereochemistry of fatty acid in acylglycerols did not influence the bioavailability of EPA and DHA.

1. Introduction

Since our original observations in Greenland Eskimos of an association between dietary intake of marine long-chain poly-unsaturated n-3 fatty acids (n-3 PUFA) and biological and cellular functions [1,2] much interest has been focused on the potential health benefits of marine n-3 PUFA and seafood in the diet [3–7]. Based on this, national heart associations and governmental bodies have recommended an increased intake of oily fish and potentially the use of n-3 PUFA supplements for prevention coronary heart disease [3]. Supplementation with various n-3 PUFA formulations has served as the primary tool for obtaining an exact dose of n-3 PUFA and to perform blinded, controlled studies. Initially, deodorized fish oils, e.g. cod liver oil (CLO) and fish body oils (FBO), were used. In these preparations, the n-3 PUFA are esterified as triglycerides (TG). Problems of patient compliance due to the relatively large amounts of such oils that have to be ingested in order to reach an appropriate dose of n-3 PUFA have prompted the development of more concentrated compounds [8]. Thus, concentrates of marine oils containing up to 30–90% of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been developed. The n-3 PUFA are generally present in these formulations as free fatty acids (FFA), ethyl esters (EE) or as re-esterified TG (rTG). The term "re-esterified" is used for products made from FBO, in which the app. 30% TG content is transferred to ethyl esters and then molecularly distilled to remove the short chain and the saturated fatty acids increasing the EPA and DHA contents to around 60%. The ethyl esters are then enzymatically reconverted to glycerides. Some conflicting results have arisen from the rather few studies that have dealt with the bioavail-ability of EPA and DHA from various concentrated n-3 PUFA formulations [9–14]. The lack of a controlled study comparing the five presently commonly used fish oil supplements (natural TG in fish body oil and CLO, EE, FFA and rTG) led us to undertake a blinded, placebo-controlled study in healthy volunteers, using generally available products. The enrichment of EPA and DHA in plasma TG, cholesterol esters and phospholipids was examined after intake of five different n-3 FA formulations or placebo oil (corn oil, CO) for 2 weeks.

2. Methods

2.1. Subjects

Seventy-two healthy subjects (36 women aged 21–56 years and 36 men aged 23–55 years) volunteered for the study.

☆The main results have been previously published in the proceedings of a workshop at the 29th yearly meeting of The European Society for Clinical Investigation: n-3 Fatty acids: prevention and treatment in vascular disease. S.D. Kristensen, E.B. Schmidt, R. de Caterina, S. Endres (Eds). Springer Verlag, London, 1995

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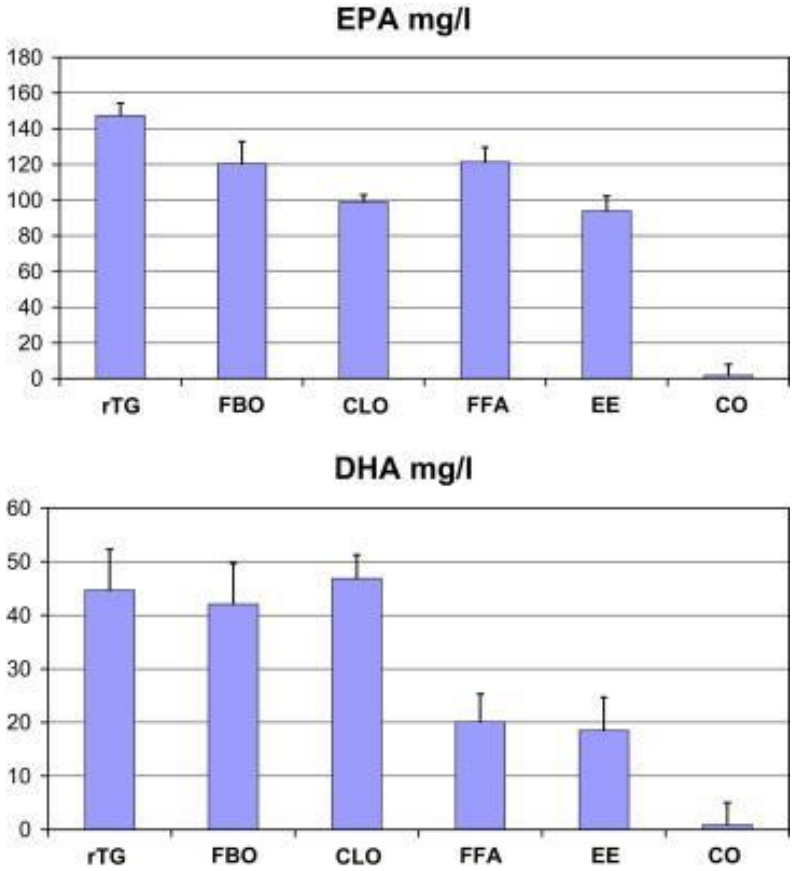
E-mail address: jdcon@post4.tele.dk (J. Dyerberg).

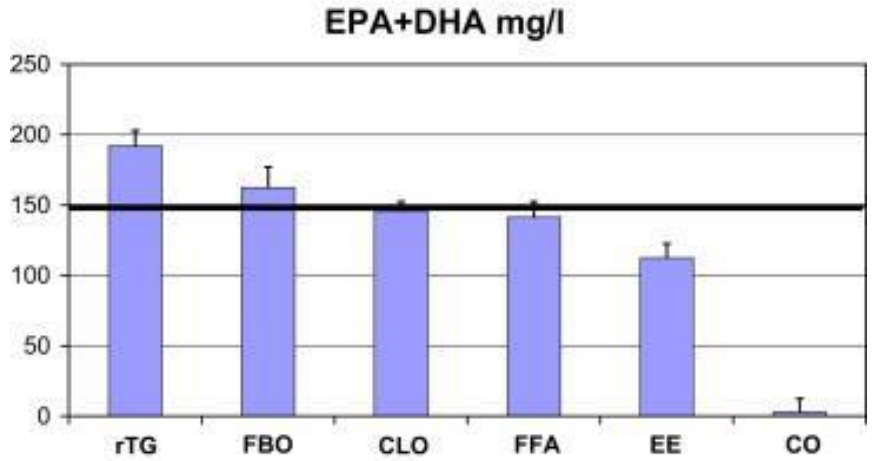
0952-3278/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.plefa.2010.06.007

- 72 subjects for 2 weeks (3.1-3.6 g/day)
  - Re-Esterified Triglycerides
  - Free Fatty Acids
  - Ethyl Esters
  - Fish Body Oil (natural TG- low potency)
  - Cod Liver Oil
  - Corn Oil

Dyerberg J, et al. Prostaglandins, Leukot Essent Fatty Acids. 2010;83(3):137-41.

Bio-availability from Dyerberg et. al.





GENOVA DIAGNOSTICS

Dyerberg J, et al. *Prostaglandins, Leukot Essent Fatty Acids.* 2010;83(3):137-41.

rTG verses EE in Omega-3 Index

European Journal of Clinical Nutrition (2011) 65, 247–254

www.nature.com/ejcn

ORIGINAL ARTICLE

Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerides versus ethyl esters

J Neubronner[1], JP Schuchardt[1], G Kressel[1], M Merkel[2], C von Schacky[3] and A Hahn[1]

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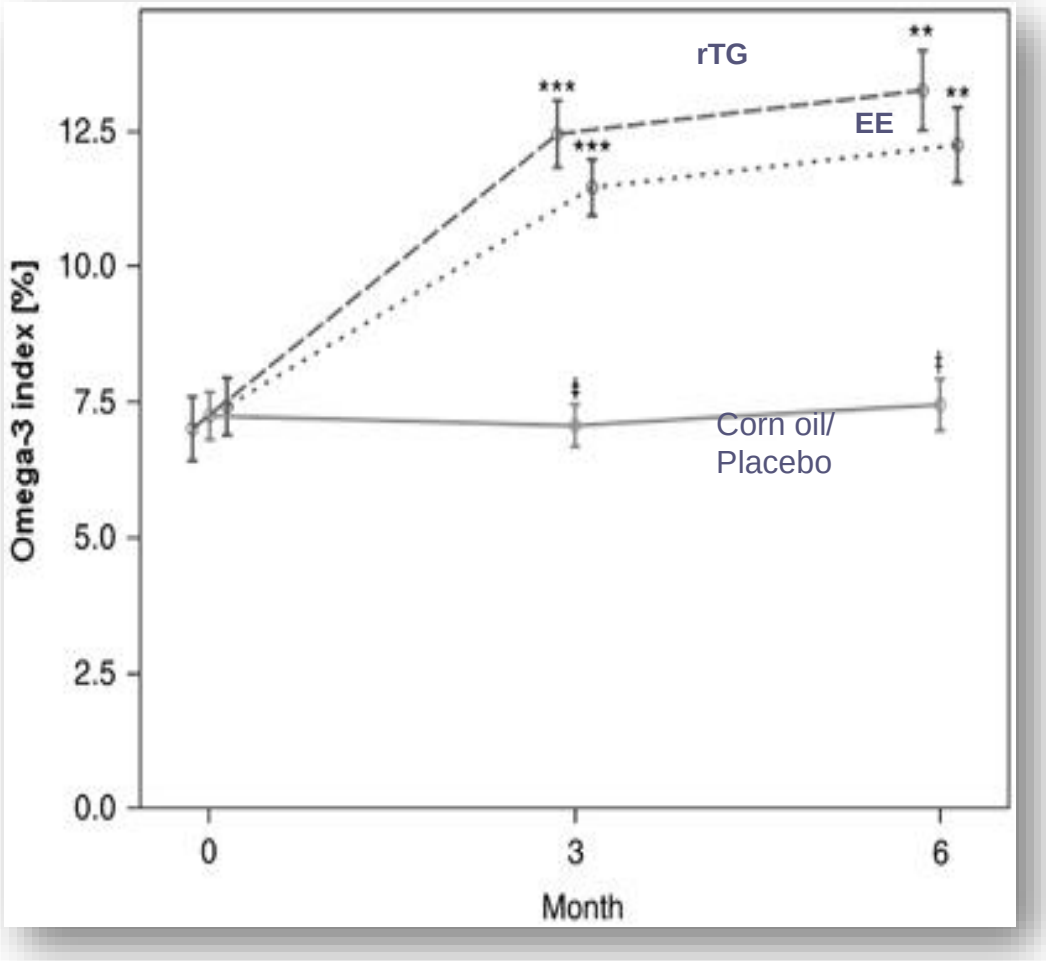
Background: There is a debate currently about whether different chemical forms of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are absorbed in an identical way. The objective of this study was to investigate the response of the omega-3 index, the percentage of EPA + DHA in red blood cell membranes, to supplementation with two different omega-3 fatty acid (*n*-3 FA) formulations in humans.
Design: The study was conducted as a double-blinded placebo-controlled trial. A total of 150 volunteers was randomly assigned to one of the three groups: (1) fish oil concentrate with EPA + DHA (1.01 g + 0.67 g) given as reesterified triacylglycerides (rTAG group); (2) corn oil (placebo group) or (3) fish oil concentrate with EPA + DHA (1.01 g + 0.67 g) given as ethyl ester (EE group). Volunteers consumed four gelatine-coated soft capsules daily over a period of six months. The omega-3 index was determined at baseline (t_0) after three months (t_3) and at the end of the intervention period (t_6).
Results: The omega-3 index increased significantly in both groups treated with *n*-3 FAs from baseline to t_3 and t_6 ($P<0.001$). The omega-3 index increased to a greater extent in the rTAG group than in the EE group (t_3: 186 versus 161% ($P<0.001$); t_6: 197 versus 171% ($P<0.01$)).
Conclusion: A six-month supplementation of identical doses of EPA + DHA led to a faster and higher increase in the omega-3 index when consumed as triacylglycerides than when consumed as ethyl esters.
European Journal of Clinical Nutrition (2011) 65, 247–254; doi:10.1038/ejcn.2010.239; published online 10 November 2010



GENOVA DIAGNOSTICS

Neubronner J, et al. *Eur J Clin Nutr*; 2011;65(2):247-54.

Prostaglandins, Leukotrienes and Essential Fatty Acids 85 (2011) 381–386

Contents lists available at SciVerse ScienceDirect

Prostaglandins, Leukotrienes and Essential Fatty Acids

journal homepage: www.elsevier.com/locate/plefa

Moderate doses of EPA and DHA from re-esterified triacylglycerols but not from ethyl-esters lower fasting serum triacylglycerols in statin-treated dyslipidemic subjects: Results from a six month randomized controlled trial

J.P. Schuchardt [a,*], J. Neubronner [a], G. Kressel [a], M. Merkel [b], C. von Schacky [c], A. Hahn [a]

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[b] 1. Medizinische Abteilung, Haus O, Asklepios Klinik St. Georg, Hamburg, Germany
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ABSTRACT

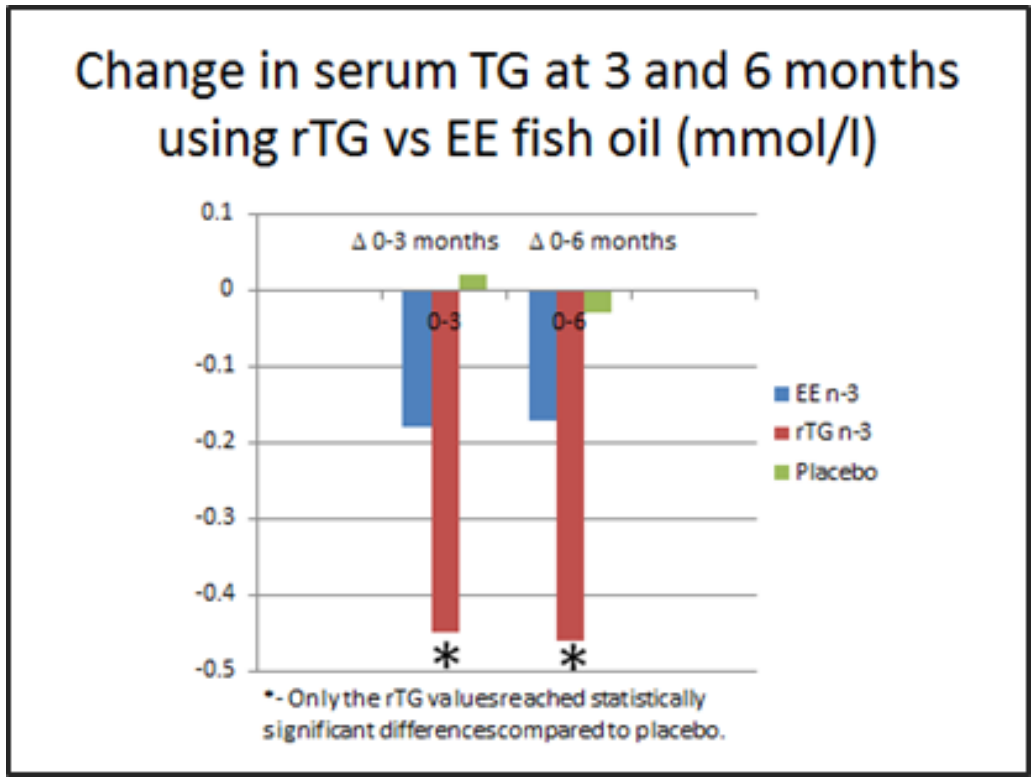
Recently, in a supplementation study over six months, it has been demonstrated that re-esterified omega-3 fatty acid triacylglycerols (n3-FA-rTAGs) led to a higher increase in omega-3-index compared to identical doses of n3-FA ethyl-esters (n3-FA-EEs), suggesting a better long-term bioavailability. The aim of this study was to examine whether differences occur between the two forms in affecting fasting serum lipid levels. 150 dyslipidemic statin-treated participants were randomized to corn oil as a placebo or fish oil either as rTAG or EE in identical doses (1.01 g EPA + 0.67 g DHA). No changes in total cholesterol, HDL or LDL levels were observed. In the rTAG-group, but not in the EE-group, fasting serum TAG levels were significantly reduced from baseline after three and six months. There was no significant difference between the two n3-FA-groups. However, serum TAG levels were significantly lowered after six months in the rTAG-group compared to the placebo-group in contrast to the EE-group.

GENOVA DIAGNOSTICS

Schuchardt JP, et al. *Prostaglandins Leukot Essent Fatty Acids*. 2011;85(6)):381-86.

Table 2
Comparison of TAG level differences between groups supplemented with omega-3 fatty acids as triacylglycerols (rTAGs) or ethyl-esters (EEs) and placebo (PP and MITT population).

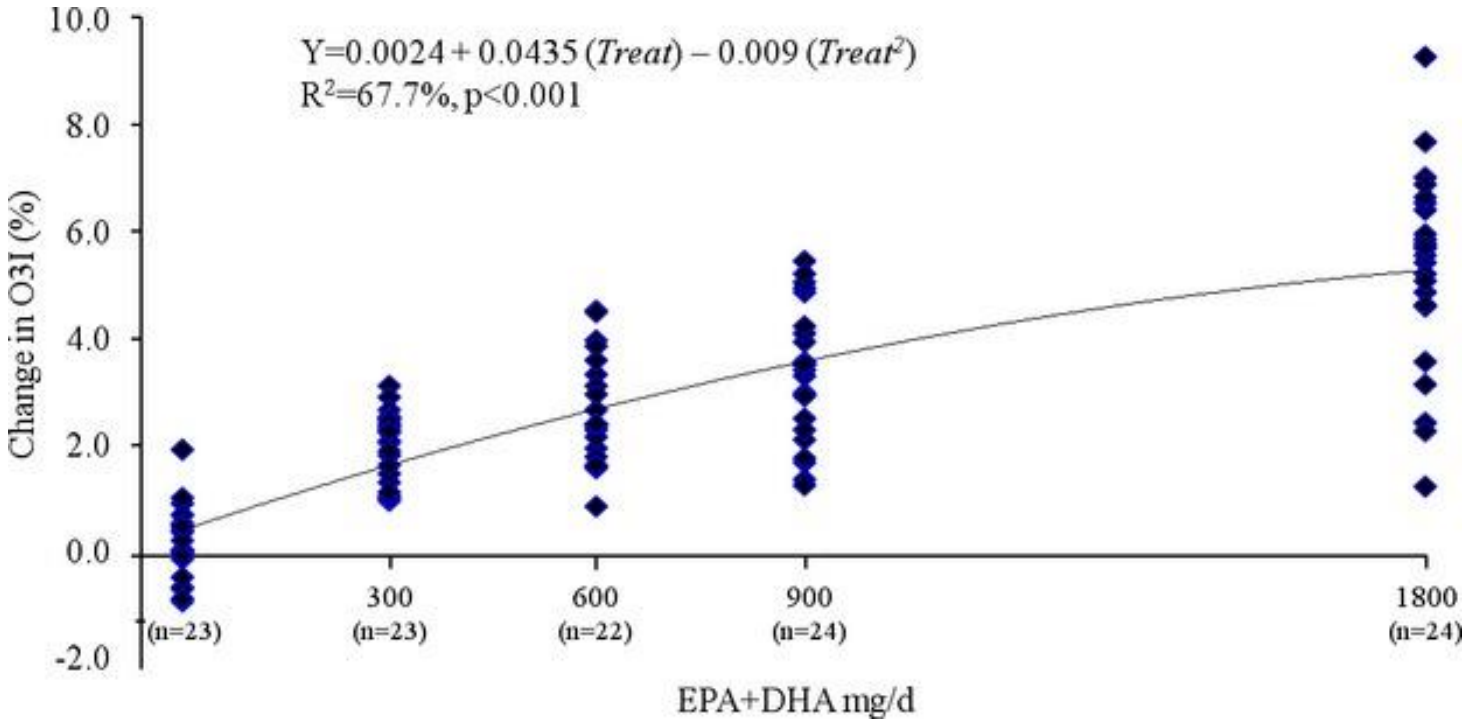
	rTAG-group		Placebo-group		EE-group	
	Mean	SD	Mean	SD	Mean	SD
PP population						
N	34		35		39	
TAG (mmol/l)						
$\Delta t_0 - t_3$	-0.31^a	0.69	0.09	0.55	−0.15	0.57
$\Delta t_0 - t_6$	-0.34^b	0.51	0.01	0.06	−0.16	0.60
MITT population						
N	52		49		49	
TAG (mmol/l)						
$\Delta t_0 - t_3$	-0.45^c	1.19	0.02	0.66	−0.18	0.60
$\Delta t_0 - t_6$	-0.46^d	1.13	−0.03	0.72	−0.17	0.76



GENOVA DIAGNOSTICS

Schuchardt JP, et al. *Prostaglandins Leukot Essent Fatty Acids*. 2011;85(6)):381-86.

Dose greatly affect average increase in O3I after 5 months



But a large individual difference in increase in omega-3 index (product was triglyceride form) is seen at all doses

GENOVA DIAGNOSTICS

Flock MR, et al. *J Am Heart Assoc.* 2013;2(6):e000513.

What about Krill Oil?

"The Influence of Bioavailability on Efficacy: When does it matter?"

- Bioavailability and Absorption are not the same thing!
- There is a general assumption that bioavailability is synonymous with efficacy and that greater bioavailability equals greater clinical efficacy
- Of course, this can be true; but there are numerous cases when this can be misleading, irrelevant, or false.
- Bioavailability of natural compounds must be understood on a case by case basis!

Absorption and Bioavailability Matter: But each substance has a different issue!

- Chondroitin Sulfate (size matters)
- Fish oil- incorporation into membranes and tissues- efficacy outcomes
- CoQ10- solubility is key
- Minerals: ionization/solubility; receptor saturation
- Botanicals:
  - Dosing issues
  - Microbiome interactions (for absorption, metabolism and efficacy)
  - First pass metabolism is extremely high for many phytonutrients

Letter

CellPress

Can improving bioavailability improve the bioactivity of curcumin?

Hong-Fang Ji and Liang Shen

Shandong Provincial Research Center for Bioinformatic Engineering and Technique, Shandong University of Technology, Zibo 255049, P.R. China

As the major yellow pigment and active chemical constituent in turmeric, curcumin has been one of the most widely studied natural products. This mainly arises from its wide-ranging pharmacological activities and healthy benefits. The efficacy and safety of curcumin in traditional Indian (Ayruvedic) and Chinese medicine for thousands of years as well as numerous clinical trials in the past decades have suggested its potential therapeutic applications in many diseases. However, curcumin has one major disadvantage: extremely low systemic bioavailability, owing to its low aqueous solubility and poor stability, has severely limited its clinical application. Numerous clinical trials found undetectable or rather low *in vivo* levels of curcumin after high doses of administration [1].

To overcome this hurdle of curcumin, researchers have recently shifted to strategies to improve its bioavailability through various delivery systems including nanoparticles, liposomes, micelles, etc. [1]. These delivery methods have been proven to significantly improve the bioavailability of curcumin compared with administration of free curcumin. Nevertheless, many studies fail to show significant improvement of the pharmacological efficacy of encapsulated curcumin in spite of improved bioavailability. Whether biotechnologies with the aim of improving the bioavailability of curcumin can produce expected improved bioactivity seems to be a complex issue and should be the focus of more attention. Among the different delivery system methods, nanocurcumin has been the most intensively studied [2]. Bisht *et al.* found that nanocurcumin is more readily dispersed in aqueous media than free curcumin, where nanocurcumin formulation had comparable efficacy to free curcumin against pancreatic cancer cell lines *in vitro* [3]. Also, Yallapu and colleagues showed that curcumin-loaded magnetic nanoparticle formulation exhibited improved uptake in cancer cells, where it was found to be as equally potent as free curcumin in suppressing breast cell growth [4]. In addition, liposomal delivery systems can also improve the stability, bioavailability, and cellular uptake of curcumin. Li *et al.* examined the antitumor activity of liposome-encapsulated curcumin and the results indicated that the activity of proliferation, apoptosis, signaling, and angiogenesis of liposomal curcumin was close to that of free curcumin [5]. The collected outcomes raise an open question: why significantly improved bioavailability of curcumin does not produce improved pharmacological efficacy in many studies?

Here, we attempt to explain the reason that enhanced bioavailability of curcumin is not associated with improved pharmacological efficacy. It has been proven that curcumin possesses low stability in aqueous solution at physiological pH or even upon addition to cultured cells, and can degrade rapidly into several bioactive molecules including ferulic acid, vanillin, etc. [6]. However, many of these bioactive degradation products have been proven to possess similar biological and pharmacological profiles with parent curcumin [7,8]. Owing to the high degradation rate of curcumin, the degradation products are expected to have high concentration in the blood circulation to achieve their pharmacological effects. Thus, different mechanisms of action may exist for free curcumin and encapsulated curcumin (Figure 1). For free curcumin, the bioactive degradation products may act as important mediators for the putative pharmacological effects because of the rather low bioavailability and high degradation rate of curcumin. Nevertheless, for nano-, liposome-, or micelles-encapsulated curcumin, the stability and bioavailability of curcumin have been significantly improved, and thus parent curcumin itself and its *in vivo* metabolites, such as curcumin glucuronide, curcumin sulfate, principally exert the pharmacological effects. When attempting to manage the bioavailability of curcumin, one should bear in mind the loss of its bioactive degradation products, and thus the overall pharmacological efficacy may not be significantly increased, despite improved bioavailability of curcumin.

This notion has gained strong support from the studies of Kurien and colleagues, in which the effects of heat and mild alkali treatment on solubility and bioactivities of curcumin were investigated [9,10]. They found that by heating a solution of curcumin to boiling for 10 min the water solubility of curcumin could be increased 12-fold [10]. It was expected that most of the curcumin would be degraded under this condition, but in fact heat had no effect on the activity of curcumin, and the heat-solubilized curcumin inhibited 4-hydroxy-2-nonenal (HNE) protein modification by 80% [9]. Additionally, although mild alkali treatment has been shown to destabilize curcumin, mild alkali (sodium hydroxide 130 mM, pH 7.6)-solubilized curcumin was interestingly found to inhibit HNE protein modification significantly [10]. Thus, despite that curcumin degraded after heat and mild alkali treatment, the bioefficacies of curcumin and its degraded formulation were comparable, which support their complementary bioactivities and the rationality of our aforementioned notion.

Corresponding authors: Ji, H-F. (jhf@sdut.edu.cn); Shen, L. (shen@sdut.edu.cn).

0165-6147/
 http://dx.doi.org/10.1016/j.tips.2014.04.001

"To overcome this hurdle of curcumin, researchers have recently shifted to strategies to improve its bioavailability through various delivery systems including nanoparticles, liposomes, micelles, etc. These delivery methods have been proven to significantly improve the bioavailability of curcumin compared with administration of free curcumin. Nevertheless, many studies fail to show significant improvement of the pharmacological efficacy of encapsulated curcumin in spite of improved bioavailability. Whether biotechnologies with the aim of improving the bioavailability of curcumin can produce expected improved bioactivity seems to be a complex issue and should be the focus of more attention……

The collected outcomes raise an open question: why significantly improved bioavailability of curcumin does not produce improved pharmacological efficacy in many studies?

Here, we attempt to explain the reason that enhanced bioavailability of curcumin is not associated with improved pharmacological efficacy.

To summarize, the different mechanisms of action for free curcumin and nano-, liposome-, or micelles-encapsulated curcumin may provide important clues to understanding why improved bioavailability of encapsulated curcumin does not lead to significantly improved pharmacological activities in many studies."

Ji HF, et al. *Trends Pharmacol Sci.* 2014;35(6):265-66.

What happens when you swallow curcumin?

- Nearly all (>95%) of the increased absorption of curcumin is quickly metabolized into glucuronide or sulphate forms.
- While some papers claim these may be active forms, most others have shown that these forms are mostly, if not completely, without bioactivity.
- Only small increases in free curcumin is noted
- Detection of other metabolites are rarely performed

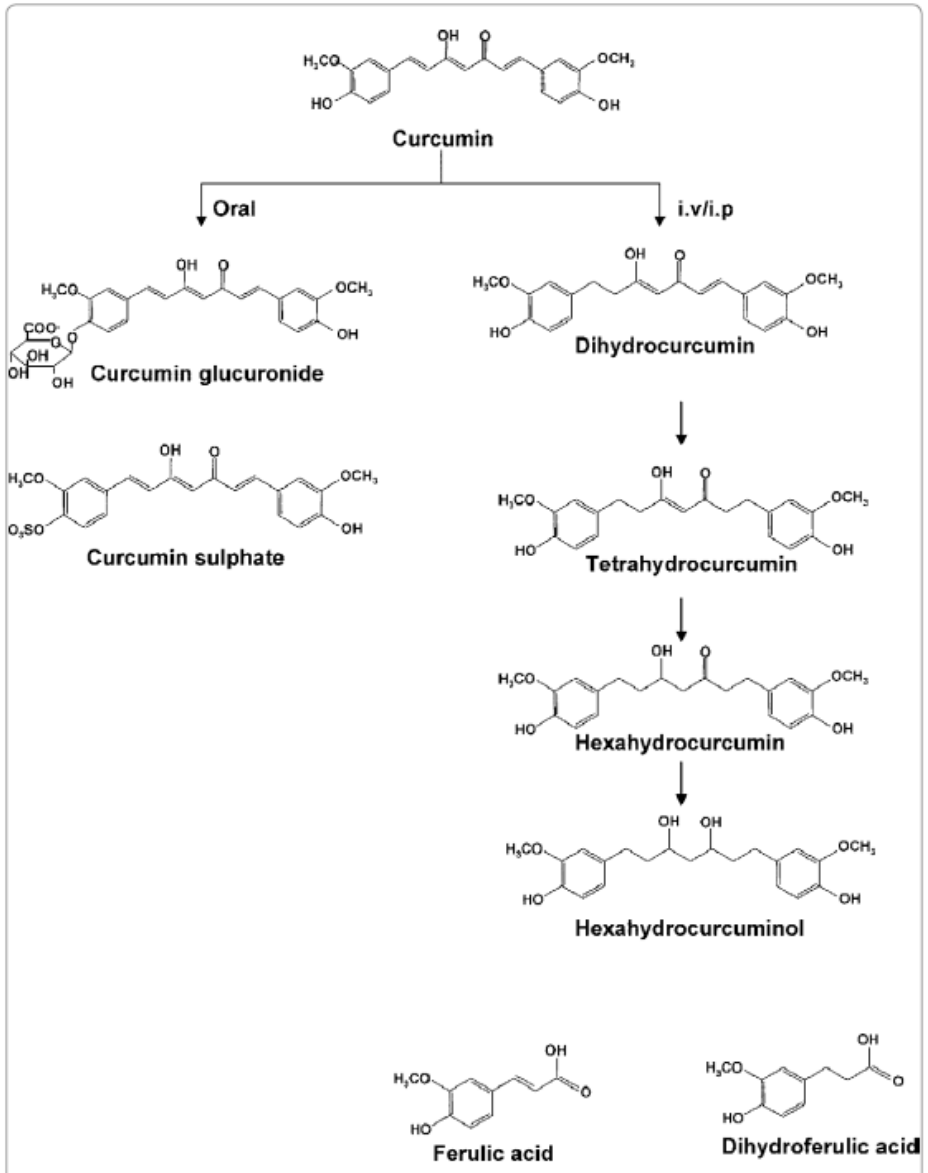


Figure 4: Metabolic pathway and possible metabolites of curcumin [1].

GENOVA DIAGNOSTICS

Prasad S, et al. *Cancer Res Treat.* 2014;46(1):2-18.

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Review

Could the gut microbiota reconcile the oral bioavailability conundrum of traditional herbs?

Feng Chen *, Qi Wen, Jun Jiang, Hai-Long Li, Yin-Feng Tan, Yong-Hui Li, Nian-Kai Zeng

Hainan Provincial Key Laboratory of R&D of Tropical Herbs, School of Pharmacy, Hainan Medical College, Haikou 571199, China

- In many cases “microbiota availability” may actually be the target of phytochemicals that are known to have poor human bioavailability
- Our desire to increase bioavailability of important phytochemicals may actually miss the very target of their therapy, or at least alter that relationship substantially

GENOVA DIAGNOSTICS

Chen F, et al. *J Ethnopharmacol.* 2016;179:253-64.

Publications in just the past few years

J Nutr Metab. 2018 Dec 16;2018:1367984. doi: 10.1155/2018/1367984. eCollection 2018.

Gut Microbiota as a Prospective Therapeutic Target for Curcumin: A Review of Mutual Influence.

Adv Nutr. 2018 Jan 1;9(1):41-50. doi: 10.1093/advances/nmx011.

The Problem of Curcumin and Its Bioavailability: Could Its Gastrointestinal Influence Contribute to Its Overall Health-Enhancing Effects?

Food Nutr Res. 2017 Aug 9;61(1):1361780. doi: 10.1080/16546628.2017.1361780. eCollection 2017.

Regulative effects of curcumin spice administration on gut microbiota and its pharmacological implications.

Crit Rev Food Sci Nutr. 2018 May 21:1-28. doi: 10.1080/10408398.2018.1478388. [Epub ahead of print]

Bidirectional interactions between dietary curcumin and gut microbiota.

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If there were only more time:

- Delivery of vitamins and minerals as capsule, tablets, powders, and liquids…..
- The GMO conundrum- some synthetics are easier to produce as GMO-free ingredients
- Do organic fruits and vegetables have higher amounts of vitamins? Minerals? Phytonutrients?
- Do we need to refrigerate probiotics?
- Do excipients like Mag. Stearate reduce absorption of supplements?
- And many many more……..

Clinicians as Educator

- The science of nutrition and the use supplements is mostly nuance—clinicians and pharmacists need to be careful when advocating a position without fully understanding the evidence for that position
- Marketers of dietary supplements will often exaggerate the "difference" between forms using limited or speculative data
- The newest form is not always better than the form it attempts to replace
- Healthcare providers using supplements must do their homework before advocating or teaching their patients or other clinicians!

Without the help of a trusted and knowledgeable healthcare provider

Most patients will try to figure it out on their own……

GENOVA DIAGNOSTICS

Thomas G. Guilliams, Ph.D.
Presenter

US Client Services: 800-522-4762

UK Client Services: 020.8336.7750

We look forward to hearing from you!

Questions?

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Understanding the Clinical Significance of the Commensal Bacteria

Christine Stubbe, ND, FABNO

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An Update: Supplementing with the Right Dietary Nutrients

Thomas G. Guilliams, Ph.D.
Founder of Point Institute,
Adj. Assistant Professor- UW-Madison School of Pharmacy
VP- Science Ortho Molecular Products

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