The Top ~20 Vitamin D Cofactors: Mechanism, Evidence Tier, and Depletion Relationships

TL;DR

- **Magnesium and vitamin K2 are the two best-supported vitamin D cofactors:** magnesium is an obligate enzymatic cofactor for every cytochrome-P450 step that makes and breaks vitamin D (backed by a human RCT plus deep mechanistic data), and vitamin K2 activates the calcium-steering proteins (osteocalcin, matrix Gla protein) whose synthesis vitamin D actually induces.
- **The evidence quality drops sharply after the top tier:** boron, zinc, vitamin A/RXR, riboflavin, omega-3, calcium and phosphate have solid mechanistic and sometimes observational support, while resveratrol, genistein/polyphenols, probiotics, glutathione, selenium, iodine, and the Gominak B-vitamin hypothesis rest mainly on in-vitro, animal, or single-lab evidence and should be labeled speculative.
- **The "cofactor depletion" concept is real for demand but oversold for true depletion:** vitamin D plausibly *raises demand* for magnesium and vitamin K (because their enzymes/proteins are consumed in D's pathway), but no rigorous human trial shows that taking vitamin D measurably drains body stores; the widely repeated "vitamin D blocks vitamin K1 uptake by 50%" claim has no traceable primary source.

Key Findings

1. **Tiering matters more than the list.** Only magnesium has direct human-RCT evidence tying it to vitamin D *metabolism* (not just co-administration outcomes). Vitamin K2's synergy is mechanistically airtight and supported by RCTs on bone/vascular endpoints, but those trials test outcomes, not D activation. Everything below these two is mechanistic, animal, in-vitro, or observational.
2. **Three distinct "cofactor" categories exist** and should not be conflated: (a) nutrients required to *make or activate* vitamin D (magnesium, riboflavin, cholesterol/7-DHC, dietary fat/bile, vitamins C and E protecting skin synthesis); (b) nutrients required for the VDR to *act* (zinc, vitamin A/RXR, magnesium again); and (c) nutrients consumed *downstream* of vitamin D action or whose demand rises with D status (vitamin K2, calcium, phosphate, magnesium, possibly pantothenic acid/B vitamins).
3. **The depletion narrative is partly evidence-based, partly folklore.** The legitimate science: D upregulates vitamin-K-dependent proteins (the MGP gene promoter contains a vitamin D response element), increasing functional K demand; and D metabolism consumes magnesium as an enzyme cofactor. The folklore: that physiological-dose D drains total-body magnesium or blocks K1 absorption by a specific percentage.

Details: The Cofactors, Ranked from Most-Established to Speculative

TIER 1 — Strongest evidence (human RCT + deep mechanism)

1. Magnesium

- **Mechanism:** Obligate cofactor for the cytochrome-P450 enzymes across the whole vitamin D pathway: 25-hydroxylases (CYP2R1, CYP27A1) in the liver, 1α-hydroxylase (CYP27B1) in the kidney, and the catabolic 24-hydroxylase (CYP24A1) and CYP3A4. Also implicated in vitamin D binding protein (DBP) transport and in VDR–RXR complex function. Because it sits on both the activating and the deactivating enzymes, magnesium acts as a *bidirectional regulator* that pushes 25(OH)D toward an optimal mid-range rather than simply up.
- **Evidence tier: Human RCT** — Dai et al., *American Journal of Clinical Nutrition* 2018;108(6):1249–1258 (NCT03265483), a double-blind 2×2 factorial trial of n=180 adults aged 40–85 nested in the Personalized Prevention of Colorectal Cancer Trial: magnesium "increased the 25(OH)D3 concentration when baseline 25(OH)D concentrations were close to 30 ng/mL, but decreased it when baseline 25(OH)D was higher (from ~30 to 50 ng/mL)." This is the only cofactor with direct human-trial evidence on vitamin D *metabolism* itself, supported by extensive in-vitro/in-vivo enzyme-dependency data. Notably, per the same paper, "according to NHANES, 79% of US adults do not meet their Recommended Dietary Allowance of magnesium."
- **Depletion relationship:** Yes — the most defensible depletion claim. Vitamin D metabolism consumes magnesium as a cofactor, so high-dose D in a magnesium-insufficient person can unmask functional magnesium inadequacy. VitaminDWiki notes the muscle-pain "reaction" to vitamin D is almost always low magnesium. (VitaminDWiki)
- **Caveats:** No RCT shows that vitamin D supplementation actually lowers serum/RBC magnesium in replete people; the "D purges magnesium" claim traces mainly to popular books and supplement blogs, not primary data. The direction of *proven* evidence is Mg→D, not D→Mg.

2. Vitamin K2 (MK-4, MK-7)

- **Mechanism:** Vitamin D increases intestinal calcium absorption and *induces the synthesis* of vitamin-K-dependent proteins — osteocalcin (binds calcium into bone matrix) and matrix Gla protein (MGP, inhibits arterial/soft-tissue calcification). These proteins are made in an inactive, uncarboxylated form; vitamin K2 is the cofactor for the γ-carboxylation that activates them. Thus D and K2 are functionally coupled: D creates the demand, K2 fulfills it. The MGP gene promoter contains a vitamin D response element capable of two- to threefold enhanced MGP expression after VDR activation.
- **Evidence tier:** Mechanistically airtight (molecular/in-vitro); animal calcification studies; and numerous **human RCTs** of combined D+K supplementation on bone density and arterial

stiffness (mostly postmenopausal women). The synergy is well-reviewed but trials test clinical endpoints, not D activation.

- **Depletion relationship:** Yes — D-induced upregulation of K-dependent proteins increases functional vitamin K demand; "excess vitamin D can induce a relative vitamin K deficiency" is a peer-reviewed mechanistic statement (van Ballegooijen et al., *Eur J Nutr* 2020), and vitamin K2 can rescue D-induced soft-tissue calcification in rats (Seyama et al., *Int J Vitam Nutr Res* 1996).
- **Caveats:** The viral "vitamin D blocks vitamin K1 uptake by ~50%" claim has no traceable primary source and appears to be an internet conflation of unrelated fat-soluble-vitamin competition studies. Human evidence that physiological-dose D causes clinically meaningful K deficiency is associational, not established.

TIER 2 — Solid mechanism, some human/observational data

3. Boron

- **Mechanism:** Extends the biological half-life of vitamin D (and steroid hormones like estradiol/testosterone), proposed to work by suppressing the catabolic enzyme 24-hydroxylase that clears 25(OH)D and by binding hydroxylated metabolites (via affinity for cis-diol groups) to shield them from degradation.
- **Evidence tier: Small human pilot studies and metabolic-ward work.** Nielsen FH et al. (1987): in 13 postmenopausal women, 3 mg/day boron after a low-boron diet nearly doubled serum estradiol (21.1→41.4 pg/mL) and testosterone (0.31→0.83 ng/mL) in low-magnesium women, and a 63-day boron-deprivation/repletion study (3 mg/d after 0.25 mg/d) increased serum 25-OH-vitamin D. A Serbian pilot study in 13 vitamin-D-deficient subjects (25(OH)D <12 ng/mL) given 6 mg/day boron as calcium fructoborate for 60 days found a significant average 25(OH)D increase of 20% (reviewed in Pizzorno, "Nothing Boring About Boron," PMC4712861). Supported by animal studies showing boron alleviates vitamin-D-deficiency dysfunctions.
- **Depletion relationship:** Not depleted by D; rather, boron is itself widely low in modern diets due to fertilizer practices. Acts to *preserve* D.
- **Caveats:** Human trials are small and often in specific populations (postmenopausal, deficient). The 24-hydroxylase mechanism is inferred, not definitively proven. Effects on serum D are modest.

4. Zinc

- **Mechanism:** The VDR is a zinc-finger protein — its DNA-binding domain contains two structural zinc ions coordinated by four cysteines, forming the two zinc-finger modules that recognize vitamin D response elements (VDREs) and enable heterodimerization with RXR. Without adequate zinc, VDR cannot bind DNA or transactivate target genes. Zinc is also needed for many enzymes and influences D's action on calcium metabolism.

- **Evidence tier:** Strong **structural/molecular biology** (crystallography, mutagenesis); **animal** data on zinc deficiency impairing D's anabolic bone action; limited human supplementation data on D status specifically.
- **Depletion relationship:** Not clearly depleted by D. VitaminDWiki notes extra zinc does not raise blood D but may help D act in tissues (via functional VDR). There is also evidence D enhances intestinal zinc absorption, suggesting a feedback loop.
- **Caveats:** The zinc-finger requirement is universal to nuclear receptors, so it is "permissive" rather than a rate-limiting lever you can push with supplementation in a zinc-replete person.

5. Vitamin A (retinol / retinoic acid → RXR)

- **Mechanism:** The VDR does not act alone — it heterodimerizes with the retinoid X receptor (RXR), whose ligand is 9-cis-retinoic acid (a vitamin A metabolite). The VDR-RXR heterodimer is what binds VDREs and drives transcription. Evidence shows RXR is a "non-silent" partner: RXR ligands can potentiate and even restore defective VDR signaling. So vitamin A status modulates the *output* of vitamin D signaling.
- **Evidence tier:** Strong **molecular/in-vitro** (heterodimerization, transactivation assays, yeast and mammalian cell systems); a hypothetical framework for combined A+D therapy in neurodegeneration; animal data. No clean human RCT isolating the A-D interaction on D outcomes.
- **Depletion relationship:** Complex/antagonistic balance rather than depletion — high vitamin A can antagonize some vitamin D actions (the classic "A vs D" balance), while adequate A is needed for full VDR-RXR function. VitaminDWiki cites work (Masterjohn) on the A-D-K balance and notes D can decrease vitamin A uptake.
- **Caveats:** The relationship is bidirectional and dose-dependent; "more A" is not simply better. Excess A can oppose D.

6. Riboflavin (Vitamin B2 → FAD/FMN)

- **Mechanism:** Riboflavin is the precursor of FAD and FMN, the flavin cofactors required by (a) cytochrome-P450 reductase, the obligatory electron-donor partner for the microsomal CYP enzymes that hydroxylate vitamin D, and (b) FAD-dependent enzymes (squalene monooxygenase, DHCR24, and CYP51A1 via P450 reductase) in the cholesterol/7-dehydrocholesterol synthesis pathway that supplies the vitamin D precursor. So riboflavin status influences both vitamin D *synthesis substrate* and *activation*.
- **Evidence tier: Mechanistic/biochemical** (flavoenzyme dependency, well-reviewed in *Advances in Nutrition* 2022, "From Cholesterogenesis to Steroidogenesis"); in-vitro enzyme studies showing FAD enhances CYP catalytic efficiency. No human trials on riboflavin→vitamin D status.
- **Depletion relationship:** Not established as depleted by D. Riboflavin declines with age and poor diet independently.

- **Caveats:** The link is inferential — riboflavin is a cofactor for the *machinery*, but no study shows correcting riboflavin raises 25(OH)D in humans.

7. Cholesterol / 7-dehydrocholesterol (7-DHC)

- **Mechanism:** 7-DHC is the direct skin precursor ("provitamin D3") — UVB photolyzes it to previtamin D3, which thermally isomerizes to vitamin D3. It sits at the branch point between cholesterol synthesis (via DHCR7) and vitamin D synthesis. Cholesterol synthesis intermediates and the enzymes around them therefore govern precursor availability. PubMed
- **Evidence tier:** Established **biochemistry/physiology** (the foundational pathway of cutaneous D synthesis); human skin-biopsy studies (LC-MS/MS) quantifying 7-DHC and its UVB conversion.
- **Depletion relationship:** 7-DHC is consumed during UVB synthesis, but human data show skin 7-DHC is *not* a limiting factor and does not differ meaningfully between younger and older adults; less than 15% converts per low-dose UVB exposure.
- **Caveats:** Supplementing cholesterol does not raise D synthesis in normal people; 7-DHC availability is rarely the bottleneck (UVB exposure, latitude, melanin, and age-related skin changes matter more). DHCR7 inhibition (genetic, as in Smith-Lemli-Opitz syndrome) actually *raises* 7-DHC.

8. Dietary fat & bile salts

- **Mechanism:** Vitamin D is fat-soluble and absorbed in the small intestine via micelles formed with bile salts and dietary fat, then packaged into chylomicrons for lymphatic transport. Adequate fat triggers bile release and micelle formation; bile salts emulsify the vitamin.
- **Evidence tier: Human controlled studies** (meal-condition studies showing fat improves D3 *absorption*); strong physiology; malabsorption-condition data (celiac, Crohn's, bariatric surgery, cholestasis impair uptake).
- **Depletion relationship:** Not a depletion relationship. Note an interesting feedback: vitamins A and D *repress* bile acid synthesis (via FGF15/19 induction and CYP7A1 suppression), a mechanism that may regulate their own absorption.
- **Caveats:** Counterintuitively, very large amounts of fat — especially polyunsaturated fat — can *reduce* D absorption; and one human study (Dawson-Hughes et al., *J Bone Miner Res* 2013) found meal fat content affected absorption but not the ultimate 25(OH)D response. "Take with fat" is sound but the effect size on status is debated.

9. Calcium

- **Mechanism:** Calcium is the principal downstream mineral vitamin D regulates (intestinal absorption, bone mineralization). It also feeds back on D metabolism: hypocalcemia

stimulates PTH, which induces renal CYP27B1 (1α-hydroxylase) to make more active 1,25(OH)2D; hypercalcemia suppresses it. So calcium status is both the target and a regulator of activation.

- **Evidence tier:** Foundational **human physiology** and abundant RCTs (D+calcium for bone). Causation well established.
- **Depletion relationship:** Inverse demand — vitamin D *increases* calcium absorption; adequate dietary calcium is needed so D's action has substrate. Low calcium with high D drives a PTH/activation response.
- **Caveats:** More is not better — VitaminDWiki and cardiovascular literature warn excess supplemental calcium (VitaminDWiki suggests keeping supplemental calcium under 750 mg/day) associates with cardiovascular risk, not stronger bones, particularly without K2 to direct it. Calcium is a cofactor to *respect*, not maximize.

10. Phosphorus / phosphate

- **Mechanism:** Co-regulated with calcium by the vitamin D/PTH/FGF23 axis. Hypophosphatemia induces CYP27B1; hyperphosphatemia and FGF23 suppress CYP27B1 and induce catabolic CYP24A1. 1,25(OH)2D stimulates intestinal phosphate absorption. Phosphate is essential for the bone mineral (hydroxyapatite) that D-driven calcium handling builds.
- **Evidence tier:** Foundational **human physiology**; FGF23-Klotho axis well characterized in humans (including CKD/hemodialysis studies).
- **Depletion relationship:** Co-regulated; D increases phosphate absorption. Not typically depleted (modern diets are phosphate-replete).
- **Caveats:** Phosphate excess (not deficiency) is the modern problem; the FGF23 feedback can suppress active D. This is a regulatory partner, not a supplement target.

TIER 3 — Mechanistic / animal / in-vitro / single-lab (speculative but plausible)

11. Omega-3 fatty acids (EPA/DHA)

- **Mechanism:** Proposed to improve membrane incorporation and absorption of fat-soluble D, exert anti-inflammatory synergy, and possibly support conversion to active 1,25(OH)2D. VitaminDWiki frames omega-3 as increasing *active* vitamin D especially in people with poor liver/kidney function.
- **Evidence tier:** Mixed — a **small pilot RCT** in hemodialysis patients (Dong-A University, NCT01596842) found a *tendency* toward increased 1,25(OH)2D with omega-3 co-supplementation; an **RCT** in obese children found DHA+D co-supplementation improved D status more than D+wheat-germ oil; anti-inflammatory mechanisms well established.
- **Depletion relationship:** Not depleted by D; both have declined in modern diets and are jointly recommended for restoration.

- **Caveats:** Human results are "tendency"-level and in special populations; bioavailability varies widely by product. Causal effect on D status in healthy people is unproven.

12. Resveratrol

- **Mechanism:** Potentiates 1,25D binding to VDR, promotes VDR-RXR heterodimerization, and activates SIRT1, which *deacetylates* the VDR — deacetylated VDR is more transcriptionally active. Synergizes with 1,25D to amplify VDRE-driven transcription in vitro.
- **Evidence tier: In-vitro** (VDRE reporter assays, mammalian two-hybrid, radiolabeled displacement) from the Jurutka/Haussler group; one **human RCT** — Bo S et al., *Nutrition & Diabetes* 2018;8:59 (PMC6147949), a double-blind trial of 192 type-2-diabetic outpatients (resveratrol 500 mg/day vs 40 mg/day vs placebo for 6 months) in which "25-hydroxy vitamin D increased in the Resv500 arm only, without between-group differences," and serum phosphorus differed significantly versus placebo (0.07 vs −0.01 μmol/L, p=0.002).
- **Depletion relationship:** None — it is an exogenous potentiator, not consumed by D.
- **Caveats:** Largely cell-culture evidence; the human RCT showed only a within-arm D increase with no significant between-group difference. Bioavailability of oral resveratrol is poor.

13. Genistein / polyphenols

- **Mechanism:** Soy isoflavones and other polyphenols modulate vitamin D metabolism by inhibiting the catabolic CYP24A1 enzyme (slowing 1,25D breakdown) and influencing VDR/CYP expression; some act on the same nuclear-receptor machinery.
- **Evidence tier: In-vitro / mechanistic** (CYP24A1 inhibition, cell studies); some animal data. No definitive human D-status trials.
- **Depletion relationship:** None established.
- **Caveats:** Polyphenols have pleiotropic, dose-dependent and sometimes opposing effects; extrapolating from cell lines to human D status is speculative.

14. Glutathione (and precursors L-cysteine / NAC)

- **Mechanism:** Glutathione status (via L-cysteine) upregulates vitamin-D-regulatory genes — vitamin D binding protein (DBP) and the hydroxylase genes CYP2R1/CYP27B1 — and raises 25(OH)D, apparently by increasing H2S and nitrite signaling and lowering oxidative stress that otherwise impairs D metabolism. VitaminDWiki calls glutathione the "master antioxidant" that improves activation of multiple vitamin D genes.
- **Evidence tier:** Predominantly **single-lab** (Sushil Jain, LSU Health Shreveport): in-vitro (human hepatocytes, renal cells, monocytes), animal (Zucker diabetic rats, high-fat-diet mice), observational human correlation in type-2 diabetes, plus one **small human RCT** — Jain SK et al., *BMJ Nutrition Prevention & Health* 2024 Aug 7;7(2):e000856 (NCT04939792):

165 vitamin-D-deficient African Americans aged 18–65 randomized to placebo, L-cysteine 1000 mg/day, vitamin D 2000 IU/day, or both; the combination significantly raised bioavailable and total 25(OH)D and reduced inflammatory markers (neutrophil-to-lymphocyte ratio, CRP).

- **Depletion relationship:** Low GSH and low 25(OH)D co-occur; epigenetic animal work suggests GSH deficiency dysregulates D-metabolizing genes. Whether D consumes GSH is not established.
- **Caveats:** Almost all evidence is from one research group and from metabolic-disease models; independent replication is limited; the one human RCT is small, single-population, short, and reports biomarkers not clinical endpoints. Direction of causation is uncertain (both may reflect underlying oxidative stress).

15. Vitamin C (ascorbate)

- **Mechanism:** Two relevant roles: (a) skin photoprotection — as an antioxidant it reduces UV-induced oxidative damage, and its level in skin is depleted by UV; combined with vitamin E it raises the minimal erythemal dose, potentially protecting the synthesis environment; (b) downstream bone action — it is the cofactor for prolyl/lysyl hydroxylase in collagen synthesis (the bone matrix that D-driven mineralization fills) and epigenetically promotes osteoblast differentiation, complementing the D-calcium-K2 triad.
- **Evidence tier: In-vitro** (osteoblast differentiation, collagen synthesis); **human** photoprotection studies (C+E raising MED — one retrospective study found combined ascorbic acid + α-tocopherol over 7 weeks raised MED by 77.6%, from 103±29 to 183±35 mJ/cm^2); observational links between vitamin C and bone density. Not a direct D-activation cofactor.
- **Depletion relationship:** Vitamin C is depleted in skin by UV exposure (the same exposure that makes D), an indirect link.
- **Caveats:** Its role is adjacent to vitamin D (bone matrix, skin antioxidant) rather than in D synthesis or activation per se. No evidence it raises 25(OH)D.

16. Vitamin E (tocopherols)

- **Mechanism:** Lipid-soluble antioxidant that protects skin membranes and 7-DHC-containing keratinocytes/fibroblasts from UV-induced oxidative damage; works with vitamin C to raise photoprotective capacity. Also a general membrane antioxidant protecting fat-soluble D and the lipid milieu.
- **Evidence tier: In-vitro and human** photoprotection studies (C+E combinations); osteoblast in-vitro work showed vitamin E (unlike C) did *not* increase matrix protein synthesis.
- **Depletion relationship:** Not depleted by D. Note high vitamin E can reduce vitamin K1 absorption (a separate interaction).
- **Caveats:** Weakest of the antioxidant trio for a direct D link; some osteoblast data were negative. Its "cofactor" status is by membrane protection, not pathway participation.

17. Probiotics / gut microbiome

- **Mechanism:** Specific probiotic bacteria (e.g., *Lactobacillus rhamnosus* GG, *L. plantarum*) increase intestinal VDR expression at the transcriptional and protein level and increase vitamin D absorption; the relationship is bidirectional, as VDR signaling shapes the microbiome. Vitamin D supplementation in turn increases gut microbial diversity and beneficial taxa (Akkermansia, Bifidobacterium).
- **Evidence tier: Animal** (VDR-knockout mice, gnotobiotic models) and **in-vitro**; a **human pilot study** showed *L. rhamnosus* GG upregulated colonic VDR in ulcerative colitis patients; a human supplementation study (n=80 vitamin-D-deficient women, *Sci Rep* 2020) showed D changed the microbiome. Systematic reviews suggest D+probiotic co-supplementation outperforms either alone.
- **Depletion relationship:** Not depletion; a reciprocal regulatory loop. (See Gominak hypothesis below for a related depletion idea.)
- **Caveats:** Much evidence is in disease models (IBD); strain-specific effects; human VDR-expression data are pilot-scale.

18. B vitamins / pantothenic acid (the Gominak hypothesis)

- **Mechanism:** Stasha Gominak hypothesizes that restoring vitamin D normalizes the gut microbiome, which is the primary source of 7 of the 8 B vitamins (especially pantothenic acid/B5, which becomes coenzyme A). In her model, vitamin D repletion can paradoxically *induce a secondary pantothenic acid deficiency* as repair processes consume B-vitamin stores, which is why she pairs D with a B-complex.
- **Evidence tier: Hypothesis / uncontrolled clinical observation** (Gominak, *Medical Hypotheses* 2016;94:103–107) plus supporting microbiome-genomics work showing gut bacteria synthesize B vitamins. Riboflavin (B2) separately has a genuine biochemical role (entry 6); B6 supports magnesium-dependent enzymes.
- **Depletion relationship:** This is the clearest *depletion-as-D-rises* claim besides magnesium and K — but it is a hypothesis, not a proven mechanism.
- **Caveats:** Published in *Medical Hypotheses* (a journal that historically did not apply conventional peer review); uncontrolled; the "D depletes B5" causal chain is unproven. B12/folate links to D are observational at best.

19. Selenium

- **Mechanism:** Selenoproteins (glutathione peroxidases, thioredoxin reductase) provide antioxidant defense and are partly vitamin-D-responsive (thioredoxin reductase was identified as a 1,25D-responsive gene in osteoblasts). Most studied in the context of D + selenium for thyroid autoimmunity (both reduce thyroid antibody titers).

- **Evidence tier: Human RCTs/pilot studies** in Hashimoto's and Graves' disease (selenomethionine + D); in-vitro identification of Se-dependent D-responsive genes. Not a D-activation cofactor.
- **Depletion relationship:** None established with D directly.
- **Caveats:** The D-selenium evidence is about thyroid/immune outcomes, not D status or activation. Selenium has a narrow safe range; supplementation should be cautious.

20. Iodine

- **Mechanism:** No direct role in vitamin D synthesis, activation, or VDR function. Linked only indirectly through thyroid physiology (where selenium and D also act) and listed among bone/mineral cofactor formulas. VitaminDWiki tags iodine among cofactors largely for whole-body mineral balance.
- **Evidence tier: Weak/indirect** — no mechanistic D link; association is via thyroid co-management.
- **Depletion relationship:** None established.
- **Caveats:** Inclusion is by convention in cofactor formulas, not by demonstrated vitamin D mechanism. This is the most speculative entry.

Honorable mentions / related factors

- **Strontium** appears in bone-cofactor formulas (Colgan/AlgaeCal-type blends) for its calcium-mimetic bone effects but has no established vitamin D pathway role.
- **UVB / sunlight** is the upstream driver of cutaneous synthesis rather than a "cofactor" per se; antioxidants (C, E, polyphenols) protecting skin during exposure are the cofactor angle.
- **PTH, FGF23, and Klotho** are the hormonal regulators (not nutrients) that gate CYP27B1/CYP24A1; they explain *why* calcium and phosphate behave as cofactors.

Recommendations

1. **Prioritize the two evidence-backed cofactors first.** For any high-dose vitamin D protocol (VitaminDWiki defines "a lot" as >4,000 IU/day sustained for more than a month), pair with **magnesium** (the single most important and the one with human-RCT support for D metabolism) and **vitamin K2** (mechanistically essential for steering D-mobilized calcium). These are the staged "must-haves."
2. **Add the Tier-2 micronutrients as low-risk, plausible support:** boron, zinc, and adequate vitamin A (from balanced diet, not megadoses), riboflavin, and ensuring vitamin D is taken with a fat-containing meal. Treat calcium with restraint (favor dietary; cap supplemental calcium) and assume phosphate is already replete.
3. **Label Tier-3 honestly as adjacent/speculative.** Resveratrol, genistein/polyphenols, glutathione/NAC, probiotics, the Gominak B-vitamin protocol, selenium, iodine, and

vitamins C/E should be presented to readers as mechanistically interesting but lacking confirmatory human trials on vitamin D status. Glutathione/L-cysteine is the most promising of these and worth a "watch this space" flag pending independent replication of the Jain lab's work.

4. **Benchmarks that would change the tiering:** promote a cofactor up a tier when an independent, adequately powered human RCT shows it changes 25(OH)D or 1,25(OH)2D (or a hard clinical endpoint) — e.g., a multi-center replication of glutathione/L-cysteine raising bioavailable 25(OH)D, or an RCT showing riboflavin repletion raises D status. Demote or flag any claim (like "D blocks K1 by 50%") that cannot be traced to a primary source.
5. **Frame the depletion concept carefully for readers:** present magnesium (consumed as an enzyme cofactor) and vitamin K (consumed activating D-induced proteins) as the defensible "rising-demand" cases, and explicitly debunk the unsourced "D depletes magnesium stores" and "D blocks K1 uptake 50%" claims.

Caveats

- **Evidence asymmetry:** Only magnesium has direct human-RCT evidence on vitamin D *metabolism*. Vitamin K2's clinical RCTs test bone/vascular outcomes, not D activation. Every other entry is mechanistic, animal, in-vitro, observational, or single-lab. Readers should not treat in-vitro VDR potentiation (resveratrol, genistein) as proof of a clinical effect.
- **"Cofactor" is used broadly here** — spanning true enzymatic cofactors (magnesium, riboflavin), structural requirements (zinc, vitamin A/RXR), substrates (7-DHC, cholesterol), absorption aids (fat/bile), downstream partners (K2, calcium, phosphate), and speculative modulators (polyphenols, probiotics). This breadth is intentional per the brief but means "cofactor" does not imply equal importance or evidence.
- **Depletion claims are the most error-prone area online.** The genuinely supported claims (Mg consumed by D's enzymes; K demand raised by D-induced proteins) are routinely exaggerated into unsupported "D drains your magnesium/vitamin K" marketing. The specific "50% K1 uptake reduction" figure has no identifiable primary source.
- **Source hygiene:** Much web content on this topic is supplement-marketing that fabricates or vaguely attributes citations and quotes influencers as if primary evidence. This report relied on PubMed/journal-indexed primary literature for mechanisms and flagged VitaminDWiki framing as the requester's own curatorial reference rather than primary evidence.
- **Individual variability:** Genetic polymorphisms (CYP2R1, CYP27B1, VDR, DBP/GC) and conditions (CKD, malabsorption, obesity) strongly modify how any cofactor behaves, which is why population-level cofactor effects are often small or bidirectional (as with magnesium).