

Perspective

Vitamin D, Calcium, and Colorectal Neoplasia: New Insights on Mechanisms of Action

Perspective on Fedirko et al., p. 213

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In 1980, Garland and Garland (1) proposed that the lower mortality rates from colorectal cancer in southern regions of the United States might be related to higher sunlight exposure in those areas via a vitamin D-related mechanism. Calcium also has modest protective effects against colorectal neoplasia (2). Many epidemiologic studies indicate a relationship between the intake of calcium and/or vitamin D and colorectal adenoma and cancer incidence (2–4); some clinical trial data support this relationship as well (described in more detail below). Vitamin D and calcium have become agents of great interest and intensive study for the chemoprevention of colorectal cancer, and the report by Fedirko et al. (5) in this issue of the journal addresses several gaps in this area of research. These gaps include the need for more evidence for effective doses of vitamin D and calcium in human chemoprevention, a better understanding of how vitamin D and calcium interact to affect colorectal cells, and the identification of biomarkers of vitamin D/calcium chemopreventive effects and pathways through which vitamin D and calcium exert these effects (6).

Several mechanisms of action for vitamin D and calcium related to chemoprevention have been studied. Vitamin D actions include induction of G₀-G₁ cell-cycle arrest (7, 8); promotion of cell differentiation, possibly via a pathway involving β -catenin (9, 10); and stimulation of apoptosis (11). Putative biological activities related to the apparently protective effects of calcium in the colon include binding of bile acids (12, 13), decreasing cytotoxicity of fecal water (14), inhibition of cellular proliferation (14, 15), and induction of apoptosis (16). Although these mechanisms have been well studied in animal models or *in vitro*, the paucity of studies of the potential modes of action of these agents within controlled studies of vitamin D and calcium supplementation in humans is an important research gap. This issue was addressed by Fedirko et al. in their translational analysis of apoptosis, an overlapping mechanism of action proposed for both vitamin D and calcium, in clinical trial specimens.

These investigators conducted a double-blind, 2 × 2 factorial clinical trial in patients randomized to receive calcium carbonate (2.0 g/d) and/or vitamin D (800 IU/d; as cholecalciferol) or placebo. They evaluated the effects of these treatments on the apoptosis markers Bax and Bcl-2 in normal colon mucosa. Bax expression increased by a statistically significant 56% along the full length of the colonic crypts among individuals treated with vitamin D (versus placebo); Bax expression increased by a statistically nonsignificant 33% in patients receiving either calcium or calcium plus vitamin D (versus placebo). Therefore, the results of this well-designed study show that vitamin D supplementation in humans has significant effects on markers of apoptosis, providing support for the biological activity of this agent. This study also addressed the need for more evidence on the minimal doses of calcium and vitamin D required for chemoprevention; this question has yet to be unequivocally clarified despite a substantial body of epidemiologic research into the relationship of these agents with cancer risk.

Previous clinical data addressing the minimal dose issue came from perhaps the best known clinical trial of combined calcium and vitamin D, which was conducted within the Women's Health Initiative (WHI; ref. 17). This large trial randomized 18,716 women to receive elemental calcium (1,000 mg/d) plus vitamin D (400 IU/d) as cholecalciferol and 18,106 women to receive matching placebo (17). After a mean follow-up of 7 years, calcium plus vitamin D had no effect on the incidence of colorectal cancer (17). However, a nested case-control study found a statistically significant inverse trend between baseline concentrations of 25(OH)D, the biomarker of vitamin D status, and colorectal cancer incidence among WHI participants overall ($P_{\text{trend}} = 0.02$; ref. 17). As might be expected, the results of the WHI trial generated several new questions, including the possibilities that women randomized to the trial already had sufficient vitamin D and calcium intake and thus would not benefit further from the study supplement, or that the WHI dose of vitamin D was too low to provide any protection against developing colorectal cancer (4, 17). Regarding the latter possibility, although WHI women in the calcium plus vitamin D arm had a 28% increase in circulating 25(OH)D concentrations 2 years postrandomization (17), it has been hypothesized that consumption of the recommended adequate intake of 400 IU/d of vitamin D would be unlikely to confer marked biological effects among people of ages 50 to 69 years (reviewed by Hollis in ref. 18). Consistent with this hypothesis, larger doses of vitamin D than that (400 IU/d) used in the WHI trial may be necessary for any measurable chemopreventive activity.

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This hypothesis gained some support from the work of Lappe et al. (19), who conducted a clinical trial of supplemental calcium (1,400-1,500 mg) with or without vitamin D as cholecalciferol (1,100 IU) for lowering fracture risk in 1,179 women. A statistically significant reduction in all-cancer incidence, a secondary end point, occurred in the calcium plus vitamin D arm (odds ratio, 0.40; 95% confidence interval, 0.20-0.82), and a marginally significant reduction occurred in the calcium-alone arm (odds ratio, 0.53; 95% confidence interval, 0.27-1.03). Although its number of cancer end-point events was relatively small ($n = 50$), this trial highlights the importance of establishing an effective dose of vitamin D and calcium for cancer chemoprevention. The work of Fedirko et al. (5) indicated that a vitamin D dose of 800 IU/d was sufficient to markedly increase Bax expression in adults of ages 30 to 75 years. Therefore, although it has been argued that >800 IU/d of vitamin D is necessary for optimal health among adults (18), the currently reported work shows that putative chemoprotective effects of 800 IU/d can be detected via biomarker measurements in a human population.

The work of Lappe et al. (19) and Fedirko et al. highlights the importance of exploring (a) the interaction between calcium and vitamin D and (b) other potentially important contributors to vitamin D/calcium pathway effects on cancer end points that have been suggested by prior work (20-23).

One such factor, estrogen, was examined in the WHI, where some women in the calcium/vitamin D treatment arm were concurrently randomized to either estrogen or placebo (23). The effect of calcium and vitamin D supplementation on outcome was modified by estrogen treatment ($P_{\text{interaction}} = 0.018$); women receiving the estrogen placebo plus calcium/vitamin D had a modest reduction in colorectal cancer risk (hazard ratio, 0.71; 95% confidence interval, 0.46-1.09; versus calcium/vitamin D placebo), and women receiving estrogen plus calcium/vitamin D had a corresponding, suggestive increase in this risk (hazard ratio, 1.50; 95% confidence interval, 0.96-2.33; versus calcium/vitamin D placebo; ref. 23). Therefore, the biological activity of both agents against carcinogenesis also very likely depends on multiple factors, including hormones and other nutrients, that affect the cellular environment. Supporting this concept is the finding of Fedirko et al. that the combination of calcium and vitamin D was not as effective as vitamin D alone in promoting Bax expression. This result is somewhat contrary to what has been described in some epidemiologic and clinical studies (19-21), which suggest that calcium and vitamin D combined may be more effective than either agent alone against the development of colorectal neoplasia. Puzzling in light of the reported proapoptotic effects of both agents, the Fedirko et al. finding suggests that calcium not only did not add to but possibly also

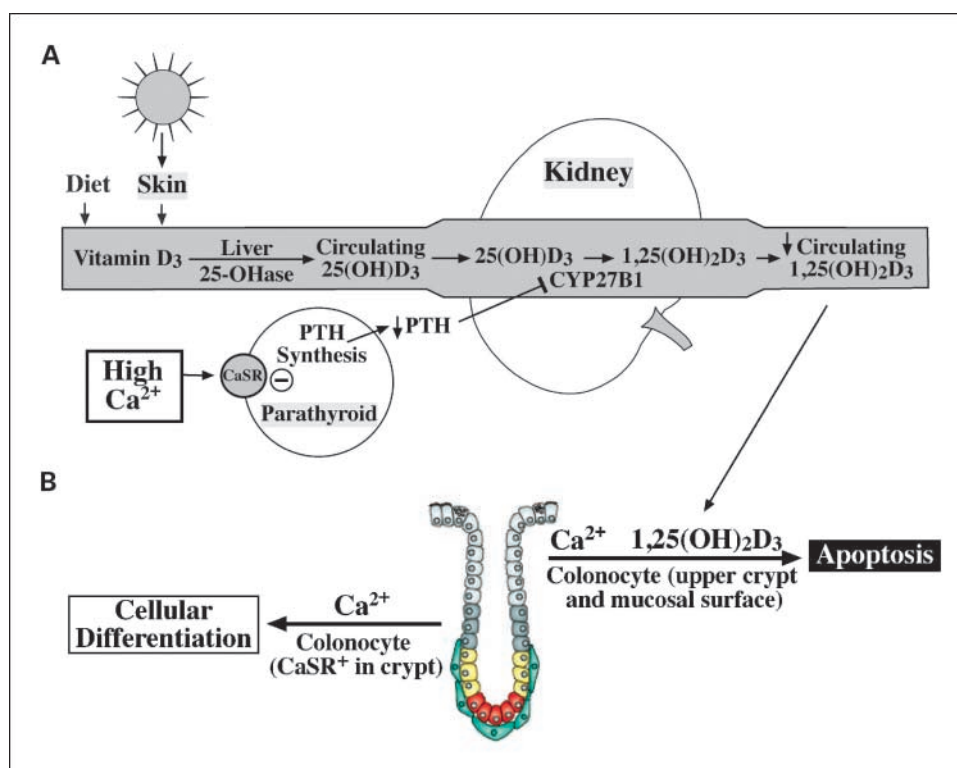


Fig. 1. Interactions between vitamin D and calcium (Ca) in colorectal carcinogenesis. *A*, regarding the intricate biological relationship between Ca^{2+} and vitamin D homeostasis, higher concentrations of Ca^{2+} inhibit the secretion of parathyroid hormone (PTH); the lack of parathyroid hormone down-regulates CYP27B1 activity, leading ultimately to lower circulating concentrations of $1,25(\text{OH})_2\text{D}_3$, the hormonal form of vitamin D. In turn, this effect would reduce the $1,25(\text{OH})_2\text{D}_3$ -stimulated apoptosis that is illustrated in *B* (the colon crypt is modified from Humphries and Wright; ref. 27). *B*, calcium has a critical role in regulating cell, or colonocyte, turnover in colon crypts. The presence of calcium may have prodifferentiative and/or proapoptotic effects, depending on the state of the cell as well as its location in the crypt. As cells move toward the lumen in normal crypts, the presence of Ca^{2+} stimulates terminal differentiation via binding to and activation of calcium-sensing receptors (CaSR). As cells reach the mucosal surface, Ca^{2+} can induce apoptosis. However, in adenomatous crypts, Ca^{2+} may actually stimulate proliferation and the production of apoptosis-resistant cells.

blunted the apoptotic effects of vitamin D. A potential explanation for this observation comes from Whitfield (24). In normal colon cells, calcium seems to have a role in blocking cellular proliferation and promoting differentiation; however, even at very early stages of carcinogenesis, calcium promotes cellular proliferation and resistance to apoptosis (24). These data suggest that calcium may exhibit a functional duality depending on the state of the colonocyte or its location in the crypt; therefore, calcium may in some cases have opposing actions to vitamin D—stimulated apoptosis (Fig. 1). A further possibility suggested by the Fedirko et al. finding is that the two nutrients may affect apoptosis at different time points. For example, the effect of vitamin D on Bax expression may be more direct and thus earlier (versus that of calcium), and the effect of calcium on this pathway may be more indirect and thus later (versus that of vitamin D) via the release of caspases or by some other regulatory event (16).

Another possibility is related to the intricate biological interactions between calcium and vitamin D, which make it difficult to study the effects of either nutrient on colorectal neoplasia in isolation (25). The hormonal form of vitamin D, 1,25(OH)₂D₃, has a critical role in calcium homeostasis (26). Parathyroid hormone is secreted as calcium concentrations decrease, resulting in increased production of 1,25(OH)₂D₃. This process in turn increases the absorption of calcium in the gut and stimulates the release of calcium from the bone,

which ultimately suppresses parathyroid hormone secretion (Fig. 1). Because of this tightly regulated feedback loop, the presence of adequate or elevated calcium in the diet may in fact suppress the synthesis of 1,25(OH)₂D₃ at the cellular level or lower the homeostatic set point for 1,25(OH)₂D₃ production, which would then attenuate apoptosis stimulated by this active vitamin D metabolite (Fig. 1). Further research should clarify how vitamin D functions in the presence of other nutrients, particularly calcium.

In summary, epidemiologic evidence provides support for calcium and vitamin D as a chemopreventive regimen for colorectal cancer (2–4, 19), and the study by Fedirko et al. (5) is a timely and important contribution of translational science to understanding the underlying biology of vitamin D and/or calcium effects in colorectal carcinogenesis. This study offers additional support for induction of apoptosis as a mechanism of action of vitamin D and provides insight into the minimum dose of vitamin D that may be required to elicit a chemopreventive response in the colon. Nonetheless, further questions remain, particularly on potential interactions between vitamin D and calcium and other factors in the cellular environment.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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