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Review

The science behind vitamins and natural compounds for breast cancer prevention. Getting the most prevention out of it

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SUMMARY

This review highlights the role of vitamins and natural compounds in breast cancer prevention, with a particular focus on Vitamin D. In the last decades, both encouraging and discouraging results about the association between antioxidant supplementation and cancer have been reported to public and scientific community. Their safe and favorable toxicity profile makes them suitable to be investigated in a preventive setting. However, a recent large meta-analysis showed that treatment with beta carotene, vitamin A, and vitamin E may increase mortality, whereas the potential roles of vitamin C and selenium on mortality need further study. Likewise, folate levels were not associated with reduced breast cancer risk in a recent meta-analysis. Several studies have shown that a high proportion of women at-risk for breast cancer or affected by the disease have deficient vitamin D levels, i.e., 25OH-D <20 ng/ml or 50 nmol/L. While the association between Vitamin D levels and breast cancer risk/prognosis is still controversial, the U-shaped relationship between 25OH-D levels observed in different studies suggests the need to avoid both deficient and too high levels. Further trials using an optimal dose range are needed to assess the preventive and therapeutic effect of vitamin D. Finally, Fenretinide, a pro-apoptotic and pro-oxidant vitamin A derivative, has shown promise in several trials and its preventive potential is being assessed in young women at very high risk for breast cancer.

Antioxidants and risk of mortality

Oxidative stress is strongly implicated in most human diseases, including cancer.[1] Observational studies found positive associations between antioxidants and improved health.[2–5] Recently, also because of exposed to intense marketing, many people are taking antioxidant supplements, believing to improve their health and prevent diseases[6–9] and many primary or secondary prevention trials of antioxidant supplements have been conducted to prevent several diseases.

Bjelakovic et al. found that antioxidant supplements, with the potential exception of selenium, were without significant effects on gastrointestinal cancers and increased all-cause mortality.[10,11] In a recent review[12] the same authors published a remarkable systematic review assessing the effects of antioxidant supplements on all-cause mortality of adults included in randomized primary and secondary prevention trials.

All trials were conducted in adults randomized to receive beta carotene, vitamin A, vitamin C, vitamin E, or selenium vs. placebo or no intervention; the antioxidants were administered singly, in combination with other antioxidants, or with other vitamins or trace elements.

Analyses were stratified according to the risk of bias: trials with adequate randomization, blinding, and follow-up procedures were considered low-bias risk trials (high methodological quality), as well as trials with ≥1 unclear or inadequate quality components were classified as high-bias risk trials (low methodological quality). Meta-regression was used to assess the effect of covariates across the trials.

Forty-seven of the 68 trials (69.1%) had low-bias risk. The remaining trials had one or more inadequate components. A total of 232.606 participants were included, for a total of 68 trials, 385 publications. The primary prevention trials were 21; secondary prevention trials were 47 including 68 167 participants mainly with gastrointestinal, cardiovascular, neurological, ocular and dermatological diseases.

In the primary prevention trials the main outcome measures were cancer incidence and mortality (cause specific and all cause); in the

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secondary prevention trials outcome measures were progression of disease and mortality. All antioxidant supplements were administered orally. Beta carotene was tested in 25 trials, vitamin A in 16, vitamin C in 34, vitamin E in 55, and selenium in 21.

The pooled effect of all supplements vs. placebo or no intervention in all randomized trials was not significant (RR, 1.02; 95% CI, 0.98–1.06). In multivariate meta-regression only two covariates were significantly associated with mortality: dose of selenium (RR, 0.998; 95% CI, 0.997–0.999; P = 0.005) and low-bias risk trials (RR, 1.16; 1.05–1.29; P = 0.005). Risk mortality was significantly increased in the supplemented group in low-bias risk trials (RR, 1.05; 95% CI, 1.02–1.08), while was significantly decreased in high-bias risk trials (RR, 0.91; 95% CI, 0.83–1.00, p for interaction = 0004). When single agents were considered, beta carotene, vitamin A and E, were significantly associated with increased mortality, especially after exclusion of high-bias trials and selenium trials. Vitamin C given singly or in combination was not significantly associated to risk mortality, even after the exclusion of high-bias risk trials and selenium trials. Selenium, after exclusion of high-bias risk trials, given singly or with other antioxidants had no significant influence on mortality.

Authors discussed possible explanations for the null/negative effect of antioxidant supplements on mortality, with the evidence that oxidative stress may also be the consequence of pathological conditions[13] and, by eliminating free radicals from our organism, we interfere with some beneficial defensive mechanisms (i.e. apoptosis, phagocytosis, detoxification).[14–16] Moreover, antioxidant supplements are synthetic and not subjected to the same rigorous toxicity studies as other pharmaceutical agents.[17] Better understanding of mechanisms and actions of antioxidants in relation to a potential disease is needed.[18]

Folate and breast cancer risk

Evidence from observational case control studies suggests that increasing dietary folate intake is associated with a reduced risk of breast cancer but large cohort studies have not found any association, and animal studies suggest that folate supplementation may promote tumorigenesis.

A meta-analysis was recently published by Lewis SJ et al.[19] to summarize the available evidence from observational studies on this issue but also on the association between a common polymorphism in a key enzyme in folate metabolism (5,10-methylenetetrahydrofolate reductase [MTHFR] gene), and breast cancer risk. A total of 26 studies (14 case-control and 12 cohort studies) were identified.

A statistically significant association between folate levels and breast cancer risk (OR = 0.91, 95% CI = 0.87 to 0.96) was found for the 13 case–control studies, while on the 9 cohort studies that measured folate intake rather than biomarkers of folate the association was not statistically significant (RR = 0.99, 95% CI = 0.98 to 1.01, for a 100 μg/d increase in folate intake). When the analysis was restricted to premenopausal breast cancer, the association was significant only for case-control studies (OR = 0.87, 95% CI = 0.78 to 0.97) even if publication bias was statistically significant (small study effect overestimated the summary estimate).

The meta-analysis of the MTHFR C677T polymorphism and breast cancer risk included a total of of 17 studies, 6373 case and 8434 control subjects. The summary odds ratio for TT homozygotes versus CC homozygotes was 1.04 (95% CI = 0.94 to 1.16). In addition, we found that the summary odds ratio for heterozygotes versus TT and CC homozygotes considered together was 1.01 (95% CI = 0.94 to 1.08).

Authors concluded with a substantial no consistent or reliable evidence to support a role of dietary folate in breast cancer prevention, especially from the cohort studies, whereas the case-control studies showed a risk reduction associated with dietary folate intake, but in this case the risk of chance, bias, measurement error, and/or confounding may be high. No evidence of an association between the MTHFR C677T polymorphism and breast cancer risk was found.

Biological effects of vitamin D

Calcitriol (1,25-dihydroxyvitamin D [1,25(OH)D]), the hormonal derivative of vitamin D, has been established since the 1980s as an antiproliferative and prodifferentiation agent, and as a proapoptotic agent and an inhibitor of cell migration, which may imply an inhibitory effect on cancer.[20] Vitamin D is indeed more like a hormone and not strictly a vitamin according to the classical criteria that an essential nutrient is a substance the body cannot synthesise in sufficient quantities itself. Also, vitamins are usually involved in biochemical reactions, while 1α,25-dihydroxyvitamin D exerts its action via VDR.

Vitamin D represents a group of fat-soluble prohormones, the two major forms of which are vitamin D_2 (or ergocalciferol) and vitamin D_3 (or cholecalciferol). Endogenous synthesis of vitamin D_3 takes place in the skin under the influence of ultra violet B (UVB) radiation. Exogenous vitamin D_2 or D_3 comes from dietary intake. The overall vitamin D intake is the sum of cutaneous vitamin D and nutritional vitamin D.

Vitamin D on its own has no physiological action. To be physiologically active, vitamin D must first be hydroxylated in the liver by the enzyme 25-hydroxylase, encoded by CYP27A1 (also called the 25-hydroxylase or 25(OH)D), into 25-hydroxyvitamin D. The 25-hydroxyvitamin D is inactive, and an additional hydroxylation in the kidney by the enzyme 1α-hydroxylase, encoded by CYP27B1 (also called 41α-hydroxylase), is necessary to produce the physiologically active vitamin D metabolite, calcitriol or 1,25(OH)D. When 1,25(OH)D is sufficiently available, the enzyme mitochondrial protein encoded by CYP24A1 metabolises the 1,25(OH)D into 1α,24,25-dihydroxyvitamin D, which is further catabolised to calcitroic acid. 25(OH)D and 1,25(OH)D are transported in serum by the vitamin D-binding protein (GC).

Optimal serum levels of 25(OH)D

In general, modern society is vitamin D-deprived compared with prehistoric humans. Hundreds of thousands of years ago, our remote ancestors lived mostly in the tropics and were exposed to strong sunlight year-round. According to researchers, vitamin D deficiency didn't appear to be a problem. As people migrated away from the equator, they got less sun. "Civilization" and urbanization made the deficiency much worse, and vitamin D deficiency reached a peak in the 18th and 19th centuries when people began moving in droves from rural areas to cities, where tall buildings blocked the sunlight. Dark-skinned people who migrate northward from low, tropical latitudes produce less vitamin D, which can adversely affect the immune system as well as the skeleton. Further factors include the increase in urbanization, where people tend to live and work indoors, as well as cultural practices that tend towards sun avoidance and the wearing of traditional clothing that covers the skin.

In fact most of vitamin D supply is provided through endogenous synthesis of vitamin D_3 upon sunshine exposure and will depend on amounts of UVB reaching earth surface, on skin surface exposed to UVB and on skin pigmentation.

Only a few foods naturally contain appreciable amounts of vitamin D to have an impact either through the form of cholecalciferol (vitamin D3) derived from animal sources, or ergocalciferol (vitamin D2), from plant food. The concentrations of 25(OH)D observed today are based on contemporary cultural norms (clothing, sun avoidance, food choices and legislation).

Vitamin D and mortality from all causes

A meta-analysis of published randomised trials s showed a significant reduction of 7% in total mortality (RR 0.93, $p < 0.05$) in healthy subjects taking vitamin D. No indication of hetergeneity nor publication bias was found. Eighty-two percent of patients received vitamin D_3 (cholecalciferol), the remaining vitamin D_2 (ergocalciferol), either orally or by injection. Average daily doses ranged from 300 IU to 2,000 IU. Treatment ranged from daily to 4-monthly, and follow-up ranged from 6 months to 7 years.[21] The Netherlands Longitudinal Aging Study examined, during a 6-year follow-up, the risk of death of 1,260 community dwelling people 65 years old or more according to serum 25(OH)D levels measured at baseline.[22] The results indicated that subjects with serum 25(OH)D levels lower than 20 ng/mL had a mortality risk associated with steadily lower levels ($p < 0.0001$).

In the Third National Health and Nutrition Examination Survey (NHANES III, USA) 13,331 adults, 20 years or older, were followed for a median of 8.7 years.[23] There were 1,806 deaths, 777 from cardiovascular disease (CVD) and 424 from cancer. Serum 25(OH)D levels below 17.8 ng/mL were associated with a 26% increased rate of all-cause mortality (95% CI: +8%; +46%). However U-shaped risk curves was also pointed out with a possible increased risk when levels are above 32.1 ng/ml.

The U-shaped association was also found in two cohort studies between 25-hydroxyvitamin D levels and colorectal or prostate cancer risk.[24,25] Furthermore the Framingham Offspring Study suggest that low as well as high 25-hydroxyvitamin D could be associated with increased incidence of cardiovascular diseases.[26]

These trends could be considered as isolated observations because upper quartiles of 25-hydroxyvitamin D levels were not higher than in other studies and the statistical power to investigate the risk at very high level is very low. However, these results may also mean that, like for many agents that were proposed for cancer chemoprevention, a high vitamin D status could be associated with an increased risk of cancer or other serious adverse event. If real, this type of dose–effect relationship would mean that increasing 25-hydroxyvitamin D could bring health benefits among subjects with low vitamin D status, while it could lead to increased risks in subjects who have a high or a very high vitamin D status before starting to take supplements.

Evidence from Vitamin D trials

The Women's Health Initiative (WHI) in the USA[27] randomized 36,282 postmenopausal women to 400 IU of vitamin D per day and 1 g of elementary calcium, or to placebo.[27,28] After a mean of 7 years' follow-up the intervention did not alter the risk of colorectal and breast cancers, or of all cancers. The negative findings of the WHI trial have been attributed to inadequate vitamin D doses, too low adherence to supplementation, too short a trial duration, or interactions between vitamin D and other substances, for example, menopausal hormone replacement therapy and calcium.[29,30]

Nonetheless, the discrepancy between observational and randomized trials points to the alternative hypothesis that vitamin D status would reflect an individual's propensity to develop colorectal cancer rather than be the cause of that cancer. This propensity would be associated with lifestyle, e.g., obesity, smoking, low physical activity and other unknown risk factors that cannot be controlled by statistical analysis.

Vitamin D and cancer risk

Results from the meta-analyses on 25(OH)D serum levels and cancer incidence, within the working group of experts, organized by the International Agency for Cancer Research, showed a significant reduction in risk for colorectal cancer comparing the highest levels versus the lowest level of 25(OH)D, with a significant dose-response effect.[31,32]

For breast cancer, the pooled estimates of 0.89 reached statistical significance. However, restricting the analysis to prospective studies (3,145 cases) yielded a SRR of 0.97 for a 10 ng/ml increase (95% CI: 0.92–1.03), whereas the SSR for the five case-control studies (3,030 cases) was 0.83 (95% CI: 0.79–0.87) ($p < 0.001$ for the difference between SRRs). These results suggest that the five case-control studies were responsible for the apparent decrease in breast cancer risk associated with increasing serum 25-hydroxyvitamin D level.

The case-control design implies that the measurement of 25-hydroxyvitamin D is done in individuals already diagnosed with cancer. Therefore, results from this study design need to be interpreted cautiously because of the potential for reverse causation, that is, low vitamin D status being a consequence, rather than a cause of the disease. For example, when symptoms are severe or during the treatment of cancer, exposure to sunlight and dietary habits are likely to change (due to hospitalizations, disability or change in lifestyle).

The "nested case-control" study is a case-control study embedded within a prospective cohort study, and serum 25-hydroxyvitamin D level is measured in archived blood samples collected several years before disease diagnosis. Therefore, in cohort and nested-case-control studies, as the blood sample is taken well before the diagnosis of cancer, it is unlikely that any association observed is due to the effect of cancer on the blood level of 25-hydroxyvitamin D.

Among the studies included, the lowest values of 25(OH)D for the upper categories in average were 34 mg/ml and the upper levels of the lowest category was 18 mg/ml.[31] Inadequate levels of circulating 25(OH)D are associated also with an increased risk and poor.

A recent study on prognostic effects of circulating 25(OH)D in a cohort of patients with early breast cancer found that deficient levels of vitamin D were associated with higher-grade tumors suggesting that the prognostic effect of vitamin D may be due, in part, to the development of higher-grade tumors in vitamin D-deficient women, consistent with a potential role of vitamin D in breast carcinogenesis.[33]

Clinical suggestions

Current efforts to assess optimal serum concentrations of 25(OH)D generally focus on bone health in older white persons, and the common definition of the optimal level has been the concentration that maximally suppresses serum parathyroid hormone (PTH). In most persons, the optimal level cannot be reached with the currently recommended intake is 200 IU and 600 IU/d for younger and older adults respectively. The total 25(OH)D serum levels, i.e. $25(OH)D_2$ plus $25(OH)D_3$, is what physicians need to be aware of in their patients.

Vitamin D deficiency is defined by most experts as a 25(OH)D level of less than 20 ng/ml (50 nmol per liter).[34–37] By these standards both the European and US populations are vitamin D insufficient or deficient.

The synthetic retinoic acid derivative fenretinide

Retinoids are natural and synthetic analogues of vitamin A. They are known to play a crucial role in cellular and tissue differentiation, because of their capability to activate and/or repress specific genes and consequently to suppress tumor promotion and modify some properties of fully transformed malignant cells.[38] Retinamides are synthetic retinoids which have been modified to extend target organ specificity, increase anticarcinogenic activity, and reduce toxicity.[39]

Fenretinide is the synthetic amide of retinoic acid N-(4-hydroxyphenyl)retinamide (4-HPR) and it was synthesized in the late 1960s. The studies on fenretinide biological activity immediately showed the preferential accumulation of this drug in the breast instead of the liver,[40] the higher activity and lower toxicity when compared to other retinoids.[41] In animal model Fenretinide demonstrated to suppress the recurrence of mammary cancer after primary tumor removal[42] and to inhibit the progression of ductal hyperplastic lesions and ductal carcinoma in situ.[43]

The mechanism of action of fenretinide is not yet completely known but it has been shown that it might exert its inhibitory effects by both receptor dependent and independent mechanisms.[44–46] The binding of retinoids to the nuclear receptors (i.e., retinoic acid receptor (RAR)-alfa, -beta and -gamma and retinoid X receptor (RXR)-alfa, -beta and -gamma), which are ligand-activated transcription factors, leads to the regulation of several cellular processes, including growth, differentiation and apoptosis.[47] Furthermore, fenretinide inhibits also the proliferation of breast cancer cells that do not express retinoic acid receptors (RARs) and more recent data demonstrated that fenretinide has a very poor affinity to this receptor class.[44] Fenretinide is responsible of both the increasing of RAR-beta expression and the decreasing of cell cycle modulators in different cancer cell lines including breast cancer cells, such as cyclins D and cyclin-dependent kinases.[48–50]

A unique feature of fenretinide is the ability to inhibit cell growth proliferation through the induction of apoptosis rather than differentiation (Fig. 1), a specific effect which is completely different from that of all-trans retinoic acid.[51,52] Fenretinide mediated apoptosis seems to be tissue specific and multiple mechanisms might operate within specific tissues.[47] Generation of reactive oxygen species (ROS) such as hydrogen peroxide and superoxide seems to be critical in mediating apoptosis in different cancer cell types.[53–55] Mechanisms specific to fenretinide as compared with other retinoids are the production of nitric oxide (NO) by nitric oxide synthases (NOS)[56,57] and sphingolipid ceramide elevation in level.[58] These mechanisms may be interrelated and, in breast cancer cells, it has been shown that NO-mediated induction of apoptosis requires mitochondrial damage, including cytochrome-c release, disruption of mitochondrial transmembrane potential and ROS generation, as well as activation of caspases.[59] If we were to take a reductionist approach, ROS production and mitochondrial membrane permeabilization could reasonably be predicted to be involved, at least in part, in apoptosis induction by fenretinide in most cell systems.[60]

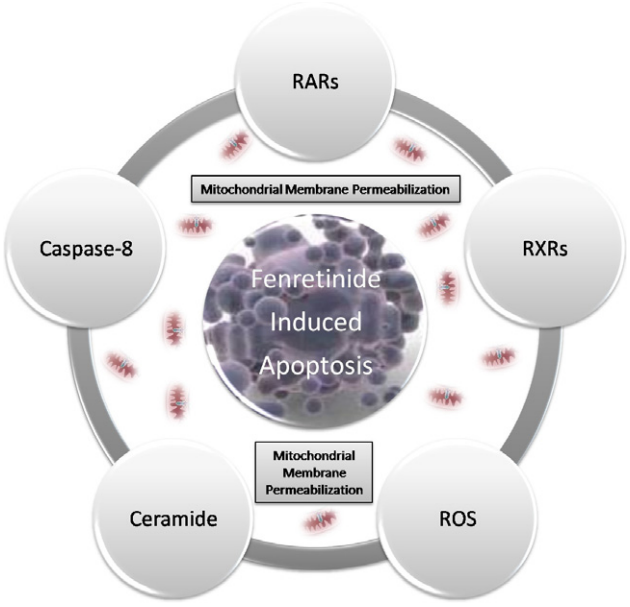

Fig. 1. Signaling pathways proposed for fenretinide-induced apoptosis. Abbreviations: RARs, retinoic acid receptors; RXRs, retinoic X receptors; ROS, reactive oxygen species.

The over fifteen-year follow up of a randomized phase III trial[61] of fenretinide to prevent second breast cancer indicates that fenretinide induced an overall 17%, durable reduction of second breast cancer incidence. More important when stratified by menopausal status, the analysis showed a 38%, statistically significant reduction of second breast cancers in premenopausal women and this protective effect persisted for up to 15 years, i.e. 10 years after treatment cessation. Importantly, the younger were the women, the greater was the trend of benefit of fenretinide, with a remarkable 50% risk reduction in women aged 40 years or younger, whereas the benefit disappeared after age 55. One explanation for the different effects of fenretinide according to menopausal status or age is a different modulation of circulating IGF-I in premenopausal and postmenopausal women, with a reduction of IGF-I levels only in premenopausal subjects. Additional fenretinide mechanisms have been investigated, such as the capability of retinoids to inhibit cell growth by reducing the expression of growth-stimulating factors or by inducing the expression of growth-inhibitory factors. In vitro, fenretinide is correlated both with a decreased secretion of insulin-like growth factor-I (IGF-I), a stimulator of epithelial cell growth, and an increased secretion of IGF-binding proteins (IGFBPs).[62,63] Higher circulating insulin-like growth factor-I levels are associated with greater risk of developing subsequent breast cancer in premenopausal women[64] and Fenretinide has shown to be able to decrease plasma IGF-1 levels.[65,66] During intervention we also observed 6 cases of ovarian cancer in the control arm vs 0 in the treated arm. At 15 years follow up, 10 cases in the control group and 6 in the fenretinide group. These results are not statistically significant but these data need to be further investigated. When considering the protective activity of fenretinide on second breast cancer in young women and a similar trend on ovarian cancer (the latter at least during intervention), it appears that young women at high risk such as those with germline BRCA-1 and BRCA-2 mutations or those with a high family risk may be ideal candidates for further investigation on this retinoid. Moreover, fenretinide is highly effective in inhibiting the growth of BRCA-1 mutated breast cancer cell lines.[67] Additionally, recent studies have shown that 4-HPR modulates gene expression in ovarian cells, with an up-regulation of expression of proapoptotic genes in OVCA433 cells and down-regulation of mutant BRCA genes in IOSE (premalignant) cells and OVCA433 cells.[68]

The prolonged effect demonstrated in the first phase III chemopreventive trial in breast cancer subjects, together with a trend of protective effect on the ovaries, has been accompanied by a very low toxicity profile (mainly reversible skin dryness and rashes and dark adaptation difficulties, often overcome by a monthly weekend suspension of the drug). Like other retinoids, fenretinide may be potentially teratogenic, although available studies show no genotoxic effects in vitro and limited effects in vivo, and a lack of storage in the human embryo. Thus, appropriate measures of contraception will be adopted when treating potentially fertile women.

Since a reduction of second breast cancer might be a surrogate marker of primary prevention, a favourable effect of fenretinide in premenopausal women provides a strong rationale for a primary prevention trial in unaffected women at high risk of breast cancer.[69] If we consider the protective activity of fenretinide on second breast cancer in young women and a similar trend on ovarian cancer, at least during intervention, it appears that women with germline BRCA 1 and 2 mutations may be ideal candidates for further investigation of this retinoid.

Key findings

Treatment with beta carotene, vitamin A, and vitamin E may increase mortality.

The potential roles of vitamin C and selenium on mortality need further study.

- By eliminating free radicals, we interfere with some essential defensive mechanisms of oxidative stress like apoptosis, phagocytosis, and detoxification.
- Antioxidant supplements are synthetic and not subjected to the same rigorous toxicity studies as other pharmaceutical agents.
- Because we examined only the influence of synthetic antioxidants, these findings should not be translated to potential effects of fruits and vegetables.

A high proportion of women at-risk for breast cancer or affected have deficient vit D levels, i.e., 25OH-D <20 ng/ml or 50 nmol/L

- Vitamin D levels and breast cancer risk/prognosis: evidence still controversial.
- U-shaped relationship between 25OH-D levels and disease (avoid deficient and too high levels).
- Importance of attaining adequate levels, i.e, 30–40 ng/ml (e.g., 100 000 IU q2 months or 10 000 IU q week).
- Further trials with optimal dose range are needed to assess the preventive and therapeutic effect of vitamin D.

Fenretinide is a retinoic acid derivative which inhibits cell growth proliferation through the induction of apoptosis rather than differentiation.

- Fenretinide might exert its inhibitory effects by both receptor dependent and independent mechanisms.
- In a phase III breast cancer prevention trial, fenretinide showed a significant reduction of second breast malignancies in premenopausal women and a promising trend on ovarian cancer during intervention. A phase III trial is underway in young women at very high risk for breast cancer.

Conflict of interest statement

The authors have no conflict of interest to declare.

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