Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms and colorectal cancer risk[1]

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Abstract

Background: Our objective was to conduct a systematic review and meta-analysis of prospective studies on colorectal cancer and vitamin D intake and 25-hydroxyvitamin D status, as part of the World Cancer Research Fund Continuous Update Project. We also aimed at conducting meta-analysis of all studies on colorectal cancer and vitamin D receptor (VDR) single-nucleotide polymorphisms.

Methods: Relevant studies were identified in PubMed (up until June 2010). Inclusion criteria were original, peer-reviewed publications, with a prospective design (for studies on vitamin D intake or status). Random effects dose-response meta-analyses were performed on cancer incidence.

Results: We observed inverse associations of colorectal cancer risk with dietary vitamin D (summary RR per 100 IU/day=0.95 95%CI: (0.93-0.98); 10 studies; range of intake (midpoints) = 39-719 IU/day) and serum/plasma 25-hydroxyvitamin D (RR per 100 IU/l=0.96 (0.94-0.97); 6 studies; range=200-1800 IU/l), but not with total vitamin D (5 studies). Supplemental (2 studies; range=0-600 IU/day) and total (4 studies; range=79-732 IU/day) vitamin D intake and 25-hydroxyvitamin D status (6 studies; range=200-1800 IU/l) were inversely associated with colon cancer risk. We did not observe statistically significant associations between FokI, PolyA, TaqI, Cdx2 and ApaI VDR polymorphisms and colorectal cancer risk. The BsmI polymorphism was associated with a lower colorectal cancer risk (RR=0.57 (0.36-0.89) for BB vs. bb, 8 studies).

Conclusions: These meta-analyses support the evidence of an inverse association between vitamin D intake, 25-hydroxyvitamin D status and the BsmI VDR polymorphism and colorectal cancer risk.

Impact: Improving vitamin D status could be potentially beneficial against colorectal cancer incidence.

Word Count: 250

Introduction

Colorectal cancer (CRC) is the third most frequently diagnosed cancer worldwide, accounting for more than one million cases and 600 000 deaths every year (1). Understanding the role of diet, a modifiable risk factor, in colorectal carcinogenesis might inform primary prevention strategies. A substantial body of literature has addressed the relationship between vitamin D and CRC risk. This relationship has been studied using estimates of dietary, supplemental and total vitamin D intakes and circulating 25-hydroxyvitamin D level, a biomarker of vitamin D status reflecting both intake and synthesis related to sunlight exposure.

Regarding dietary vitamin D intake, the World Cancer Research Fund and American Institute for Cancer Research (WCRF/AICR) report in 2007 concluded that the evidence vitamin D protects against risk of CRC was limited suggestive (RR for 100 IU/d=0.99 95%CI=(0.97-1.00)) (2). Since then, five new prospective cohort studies on vitamin D intake and CRC have been published (3-7), substantially increasing the evidence base available, but no updated dose-response meta-analyses have been published on vitamin D intake. In 2009, Huncharek et al. performed a highest versus lowest meta-analysis of vitamin D intake and CRC and observed no statistically significant results (8).

Regarding serum/plasma 25-hydroxyvitamin D status and CRC risk, three dose-response meta-analyses have been published (9-11), suggesting an inverse association. Since the most recent meta-analysis, conducted by IARC in 2010 (9), new results from the Multiethnic Cohort (12) have been published. None of these published meta-analyses provided information on proximal and distal colon cancer subtypes. In addition, these articles did not investigate a potential non-linear dose-response relationship between 25-hydroxyvitamin D and CRC risk. This could be useful for determining if an optimal value for vitamin D status can be retained regarding CRC prevention, and/or for validating optimal levels proposed by some authors (13, 14).

The vitamin D receptor (VDR) is an intracellular hormone receptor that specifically binds the biologically active form of vitamin D (1,25-dihydroxyvitamin D) and interacts with specific nucleotide sequences of target genes to produce a variety of biologic effects (15). It has been hypothesized that for individuals with similar vitamin D intake or status, those having a less active VDR could present an increased susceptibility to colorectal cancer risk. However, the evidence to date has been inconclusive. Two reviews (16, 17) and two meta-analyses (18, 19) have been published on the topic. Since the publication of the most recent meta-analysis (18), several new studies have been published (20-22), including results from the EPIC study, based on more than 1200 CRC cases. In addition, this meta-analysis focused on BsmI and FokI polymorphisms only; it did not observe overall statistically significant associations (18).

Our objective was to conduct a systematic review and meta-analysis of prospective studies on CRC and vitamin D intake published up to June 2010, as part of the WCRF Continuous Update Project. We also conducted meta-analyses of prospective studies on CRC and 25-hydroxyvitamin D level, as well as studies on VDR single-nucleotide polymorphisms. This paper provides a complete and updated state of the art regarding vitamin D and CRC risk, including substantially increased evidence base since previous reviews, and complementary types of exposures (intake / biomarker / VDR polymorphisms). It includes a linear dose-response approach (key feature in the discussion of causality), as well as an investigation of a potential non linear dose-response trend for vitamin D status, which has never been meta-analyzed before.

Subjects and Methods

Search strategy and selection criteria

The present review is part of the Continuous Update Project implemented by the WCRF/AICR and conducted at Imperial College London on the associations between food, nutrition, physical activity and the prevention of cancer. The complete protocol for the review is available on the WCRF website

(http://www.dietandcancerreport.org/cu/). Briefly, we updated the systematic literature review (http://www.dietandcancerreport.org/downloads /SLR/Colon_and_Rectum_SLR.pdf) with study results published through June 2010. We searched PubMed without any language restriction using the same search strategy that was used to retrieve papers for the WCRF/AICR report (2). The search terms (MeSH terms and text words) identified a broad range of factors on diet and nutrition. The full search strategy is available online (http://www.dietandcancerreport.org/cu/). We also hand-searched reference lists from retrieved articles, reviews and meta-analysis papers on the related topic. The search and data extraction of articles published up to June 2006 was conducted by several reviewers at Wageningen University, The Netherlands, during the systematic literature review for the WCRF/AICR report (2). The search, data selection and extraction from June 2006 to June 2010 were done by two reviewers at Imperial College London.

Studies were included in this review if they reported original data on the association of colorectal, colon or rectal cancer incidence with vitamin D intake (dietary, supplemental, total), 25-hydroxyvitamin D status and VDR single-nucleotide polymorphisms and if they were based on a prospective design (cohort or nested case-control), for studies on vitamin D intake and status. For VDR polymorphisms, all nested case-control and case-control studies were included. Only published peer-reviewed studies were included. To include the studies in the meta-analyses, estimates of the relative risks with the 95% confidence intervals had to be available in the publication. For the dose-response analysis, a quantitative measure of exposure and the number of cases and person-years were also needed. When multiple papers on the same study were identified, the inclusion of results in the meta-analysis was based on longer follow-up, more cases recruited and completeness of the information required to do the meta-analyses.

Data extraction

For each relevant study, information on study characteristics, cancer site, description of exposure, results, and details of the adjustment for confounders were extracted and stored in a database. The search,

data selection and extraction were done by two reviewers. Ten percent of the work was double checked by an independent reviewer.

Statistical analyses

Random effects models, that consider both within-study and between-study variation (23) were used to calculate summary RRs and 95% CIs for the associations of colorectal, colon or rectal cancer incidence with vitamin D intake, 25-hydroxyvitamin D level, and VDR single-nucleotide polymorphisms: FokI (rs2228570), BsmI (rs1544410), PolyA (rs17878969), TaqI (rs731236), Cdx2 (rs11568820), ApaI (rs7975232). We used the most fully adjusted RR in the paper, provided they were not adjusted for factors potentially in the causal pathway.

For vitamin D intake and biomarkers, linear dose-response, as well as highest vs. lowest meta-analyses were conducted (23). We used the method described by Greenland and Longnecker (24) for the dose-response analysis to compute the trend from the correlated RRs and CIs across categories of exposure. We estimated, using standard methods (25), the distribution of cases or person-years in studies that did not report these and reported results by quantiles. In two studies (7, 26) in which the results were reported by functional categories and person-years by category were not reported, we used variance-weighted least squares regression to estimate the trends.

The median level of exposure in each category was assigned to the corresponding relative risk when reported in the study. If not reported, the value assigned was the mid-point of the lower and upper bound in each category. For extreme open-ended categories, half the width of the adjacent exposure category was subtracted (for the lowest category) or added (for the uppermost category) to obtain the mid-point. For studies that reported results separately for colon and rectal cancer, but not combined (27-30), we combined the results using the Hamling procedure (31) to obtain an overall estimate for CRC; the same method was applied for distal and proximal colon cancer, to obtain an overall estimate for colon cancer (4).

Statistical heterogeneity between studies was assessed by the Cochran Q test and the I^2 statistic (32). I^2 values of approximately 25%, 50% and 75% are considered to indicate low, moderate and high heterogeneity, respectively. We also conducted linear meta-regression and stratified analyses by gender, number of cases, geographic location, ethnicity, range of exposure, adjustment for confounding factors such as calcium intake and sunlight exposure/season, and deviation from Hardy-Weinberg equilibrium (for studies on VDR polymorphisms) to investigate potential sources of heterogeneity. Small study bias such as publication bias was examined in funnel plots and by Egger's test (33). The influence of each individual study on the summary relative risk was examined by excluding each in turn and pooling the rest.

A potential non-linear dose-response relationship between dietary vitamin D intake and 25-hydroxyvitamin D status and CRC was examined by using fractional polynomial models (34).

A two-sided $p<0.05$ was considered statistically significant. All analyses were conducted using STATA version 9.2.

Results

Figure 1 presents the flowchart for study selection. We identified a total of 50 publications that examined the relationship between vitamin D intake and/or status (prospective studies) or VDR polymorphisms and CRC. Among these, eight publications were excluded from the meta-analyses: one was a component study of a multi-center cohort (35), two were superseded by more recent publications (36, 37), one restricted to cancer mortality as only outcome (38), one focused on VDR single-nucleotide polymorphisms that were not found in other publications on CRC risk (39) and three publications did not provide sufficient data to be included in the meta-analyses (22, 40, 41). Regarding the later three publications, only mean exposure data was provided in two of them: mean dietary vitamin D intake was either higher in non-cases than in CRC cases (40) or similar in both groups (41). The third publication

provided ORs of associations between CRC risk and heterozygous or homozygous mutant (grouped, but not separated) versus wild type, for several VDR single-nucleotide polymorphisms. No association was observed for the main VDR polymorphisms studied (i.e., BsmI, TakI and Cdx2) (22). Finally, 42 publications have been included in the present meta-analyses on CRC incidence. Online supplementary Appendix 1 provides descriptive information on these studies.

Otherwise mentioned below, there was no indication of publication bias with Egger's test and sensitivity analyses excluding one study at a time did not substantially modify the findings. For vitamin D intake and status, results of dose-response meta-analyses are presented below, whereas results of highest versus lowest meta-analyses are presented in online supplementary Appendix 2.

Vitamin D intake

We observed a statistically significant inverse association between dietary vitamin D and CRC risk (Table 1, Figure 2A): summary RR=0.95 (95%CI: 0.93-0.98), for an increase of 100 IU/day (10 studies included). Associations did not reach statistical significance for colon and rectum cancers separately (Table 1), nor for proximal and distal colon (data not shown). No statistical heterogeneity was detected except for rectal cancer, which was partly related to gender, as shown by meta-regression analysis (P=0.002). In stratified analyses, studies including more than 50% of women (5, 42, 43) showed a statistically significant inverse association between dietary vitamin D intake and rectal cancer (RR=0.78 (0.67-0.90)), whereas studies including more than 50% of men (4, 26, 44) showed no association (RR=1.09 (0.84-1.40)) (data not tabulated). Available data was insufficient to conduct separate meta-analyses by gender. Main sources of dietary vitamin D (i.e. dairy products (26, 42), fish (4, 44), or both (5, 43)) varied across studies. In the rectal cancer analysis, a higher RR (2.22 (0.99-4.97)) was observed for the Finnish Social Insurance Institution's Mobile Clinic (44) compared to other studies. In the corresponding publication (44), the authors stated that fish was the main contributor to dietary vitamin D intake, and that an increased CRC risk was associated with high consumption of salted and smoked fish in

this study. When this study was excluded from the analysis, the summary RR became 0.88 (0.77-1.02), and heterogeneity was reduced, but remained moderate (I^2=45.7%, *P*=0.12). Restriction of the analyses to studies investigating both colon and rectum cancer sites did not modify the results (summary RR for colon became 0.97 (0.91-1.03) and was unchanged for rectum). There was no strong evidence of non-linearity of the association between dietary vitamin D intake and CRC risk (p for non-linearity = 0.4), within the studied range of intake (midpoints of lowest and highest categories: 40-720 IU/d).

No dose-response analysis could be performed for supplemental vitamin D and overall CRC due to insufficient data. However, two studies were available for dose-response meta-analysis of supplemental vitamin D and colon cancer specifically (27, 28), leading to a statistically significant inverse association (summary RR per 100 IU/day=0.93 (0.88-0.98)).

The association between total vitamin D and CRC (Table 1, Figure 2B) was not statistically significant, with high heterogeneity and lower number of available studies compared to dietary vitamin D (five vs. ten). In sensitivity analyses excluding each study in turn, the summary RR for total vitamin D and CRC became statistically significant (0.97 (0.95-0.99)) and heterogeneity was substantially reduced (I^2=36.5%, *P*=0.2) when the Women's Health Study (45) was excluded from the analysis (data not tabulated). In sensitivity analyses restricted to the publications presenting results on both dietary and total vitamin D and CRC (7, 26, 42, 45) summary RRs were 0.93 (0.89-0.98) for dietary vitamin D and 0.99 (0.95-1.02) for total vitamin D. We observed an inverse association between total vitamin D and colon cancer risk (RR per 100 IU/day=0.93 (0.90-0.98), but no association for rectal cancer (Table 1, Figure 2B). In highest versus lowest meta-analyses, total vitamin D was inversely associated to both CRC 0.84 (0.72-0.97) and colon cancer 0.71 (0.58-0.87) risk (Supplementary Appendix 2).

25-hydroxyvitamin D level (biomarker of vitamin D status)

We observed an inverse association between circulating 25-hydroxyvitamin D level and CRC risk (Table 1, Figure 2C) (RR per 100 IU/l=0.96 (0.94-0.97)). Results were borderline significant for colon

cancer (Table 1). We observed an inverse association between serum/plasma 25-hydroxyvitamin D and distal colon cancer (RR per 100 IU/l=0.91 (0.85-0.98), no heterogeneity: I^2=0%, *P*=0.9, 3 studies included (46, 47), data not tabulated). Results were not statistically significant for proximal colon (data not shown) and rectum cancers (Table 1). In highest versus lowest meta-analyses, 25-hydroxyvitamin D level was also inversely associated to CRC risk 0.66 (0.52-0.84) (Supplementary Appendix 2).

There was no strong evidence of non-linearity of the association between 25-hydroxyvitamin D and CRC risk (p for non-linearity = 0.087). The curve (Figure 3) suggested that increasing 25-hydroxyvitamin D level was associated with a decreased risk of CRC in a linear dose-response manner, though a slight inflexion of the decrease in risk around the value of 1000 IU/l (24 ng/ml) could be suspected. The range of intake used in this analysis was 200-1800 IU/l (midpoints of lowest and highest categories).

Vitamin D receptor polymorphisms

The polymorphisms most often reported were BsmI and FokI. The BsmI polymorphism was associated with a lower CRC risk (RR for BB vs. bb=0.57 (0.36-0.89), 8 studies), with high heterogeneity (Table 1, Figure 4). The heterogeneity may be attributed to one study (48), for which deviation from Hardy-Weinberg equilibrium was observed. When we excluded it from the analysis, statistical heterogeneity was not detected (I^2=0%, *P*=0.8) and the inverse association persisted, although weakened (RR for BB vs. bb=0.89 (0.81-0.98)). In the publication of Park et al. (49) no CRC case and only one control presented the BB genotype, thus, it was not possible to use those results in the meta-analysis. However, this study was included in the Bb vs. bb analysis (summary RR=0.81 (0.64-1.02), 9 studies). When this study (49) was excluded, the summary RR for Bb vs. bb became statistically significant: 0.77 (0.61-0.98) (data not tabulated).

We did not observe any statistically significant association for FokI VDR polymorphisms on ten studies (Table 1). Study results were highly heterogeneous. Results by gender were not provided in the

publications, thus separate meta-analyses on men and women were not possible. However, for ff and CRC, the only study including a higher proportion of women than men (50) showed a statistically significant positive association (RR=1.84 (1.15-2.94)), whereas studies including a higher proportion of men than women (19, 48, 51) or an equal proportion of men/women (21, 49, 52-55) showed no association (RR=0.95 (0.81-1.11), I^2=33.1%, *P*=0.2 and 1.00 (0.49-1.03), I^2=86.2%, p<0.0001, respectively) (data not tabulated). For ff and colon cancer, the risk ratios also increased with the proportion of women in the study: more men than women (56): 0.71 (0.57-0.87), equal proportion of men/women (21): 1.13 (0.80-1.58) and more women than men (50, 57): 2.0 (1.32-3.03). However, for this analysis on ff and colon cancer, Egger's test (*P*=0.01) and funnel plot suggested a publication bias (i.e., inverse relationship between RR and study size).

Five studies investigated TaqI (20, 49, 52, 55, 58) and Apa I (19, 20, 49, 52, 58) polymorphisms and CRC risk. No association was observed (Table I). From these, 4 studies also reported on BsmI (19, 20, 49, 52) and 4 on Fok I (19, 49, 52, 55). High heterogeneity in the analyses on TaqI (tt vs. TT) and CRC was due to one study (55) with very small number of cases (n=26). No heterogeneity was detected when this study was excluded from the analysis (summary RR=1.07 (0.82-1.39), I^2=0%). High heterogeneity was also observed in the analysis of ApaI (AA vs. aa) and CRC. Although ethnicity was not statistically significant in meta-regression (*P*=0.11), probably due to low statistical power, restriction to studies on Caucasian populations (19, 20, 52, 58) substantially decreased heterogeneity (RR=0.84 (0.68-1.02), I^2=29.6%, *P*=0.2). RR of the study on a non-Caucasian (Asian) population was 2.22 (1.12-4.40) (49).

Four studies or less were identified on the PolyA and Cdx2 VDR polymorphisms. No association with CRC or colon cancer was observed, except a borderline significant positive association for Cc versus cc Cdx2 polymorphism and CRC (Table 1).

Discussion

In dose-response meta-analyses, we observed inverse associations between dietary vitamin D and CRC risk and between supplemental and total vitamin D and colon cancer risk. The WCRF/AICR report in 2007 concluded a limited suggestive decreased risk of CRC for foods containing vitamin D (2). The present meta-analyses, including new results from five prospective cohort studies, add to the evidence for an inverse association between vitamin D intake and CRC risk. In a recent report (59), the American Institute of Medicine (IOM) has set at 600 IU/d the Recommended Dietary Allowance for vitamin D intake for most North Americans, except for people age 71 and older, who may require 800 IU/d. These recommendations were mostly based on the role of this nutrient in bone health. The order of magnitude of vitamin D intake studied in prospective observational studies on CRC and thus included in this dose-response meta-analysis (maximal dose around 730 IU/d for total vitamin D), is consistent with IOM recommendations.

Vitamin D status depends on intake from the diet and supplements but also on synthesis in the skin under the influence of sunlight. Thus, we also analyzed vitamin D status to obtain a better picture of the relationship between vitamin D and CRC risk. Consistent with results on vitamin D intake, we observed inverse associations between circulating 25-hydroxyvitamin D and colorectal and colon cancer. These findings update those of previous meta-analysis on 25-hydroxyvitamin D and CRC (9-11), suggesting the existence of an inverse association.

The associations between polymorphisms in the VDR gene and CRC risk have been investigated in several publications, with inconsistent results, possibly because single studies may have lack statistical power. Except for BsmI and FokI, published studies on CRC and other VDR polymorphisms are scarce. The available evidence suggests that the BsmI polymorphism (BB) may be associated with a lower CRC risk. There was no statistical evidence of publication bias. This association, which strengthens the

evidence of the role of vitamin D in the etiology of CRC, requires confirmation in other studies. Beyond the potential effect of single-nucleotide VDR polymorphisms considered separately, their association in haplotypes (i.e. combinations of statistically associated single-nucleotide polymorphisms) could play an important role in the etiology of CRC (17). Interactions between the VDR gene and other genes have also been suggested. For instance, the androgen receptor gene could interact with the Fok1 VDR polymorphism, as well as with sunlight exposure and vitamin D intake (60).

The question of the existence of an optimal vitamin D status is essential for medical practice and public health. In a meta-analysis performed in 2007 (10), Gorham et al. observed that a 50% lower risk of CRC was associated with a serum 25-hydroxyvitamin D level ≥1400 IU/l (33 ng/ml), compared to <509 IU/l (12 ng/mL). Bischoff et al. (13) suggested that for several health outcomes (bone mineral density, colorectal cancer, among others), the most advantageous serum concentrations of 25-hydroxyvitamin D may be over 1272 IU/l (30 ng/mL) and probably in the range of 1527-1697 IU/l (36-40 ng/mL). The IOM committee recently stated that 20 ng/mL was the level needed for good bone health for practically all individuals (59). In an analysis including 30 studies reporting any adverse effect of high serum 25-hydroxyvitamin D in adults, no reproducible toxicity was detected below 100 ng/ml (61). However, an increased risk at high levels (≥40 ng/ml) has been suggested for pancreatic cancer (62), and the potential for a J- or U-shaped association between vitamin D status and prostate and esophagus cancers has been suggested (63, 64). Thus, the precise optimal level of 25-hydroxyvitamin D remains to establish. Our data suggest that CRC cancer risk decreases with increasing levels of circulating 25-hydroxyvitamin D in a linear dose-dependent manner (at least within the 200-1800 IU/l range studied), although risk reduction could increase less rapidly above 1000 IU/l (24 ng/ml). However, since the range of 25-hydroxyvitamin D levels is limited in observational studies, information on high 25-hydroxyvitamin D levels in association with CRC risk remains scarce and needs further research.

Several factors (hormonal, anthropometric, dietary, environmental, etc.) have been suggested to interact with vitamin D on the risk of CRC. First, in a re-analysis of the Women's Health Initiative Dietary Modification randomized control trial, a non-significant increased CRC risk was observed with the vitamin D/calcium supplementation among those who received estrogen therapy; whereas non-significant reduced risk was observed among the placebo group of the estrogen trial (65), suggesting that estrogen therapy could interact with vitamin D/calcium on CRC risk. Second, Lagunova et al. suggested that the direct relationship between obesity and CRC risk could be partly mediated by a decrease of 25-hydroxyvitamin D level with increasing body mass index (66). Next, it has been suggested that vitamin D and calcium may interact and that both may be required to decrease cancer risk (67). However, vitamin D remains associated with lower risk even after adjustment for calcium intake in several studies (47, 68), which is in favor of an independent effect of vitamin D. Nevertheless, the joint effect of both nutrients could be stronger that the sum of each independent effect (69). In the Health professionals follow-up study (47), the inverse association between 25-hydroxyvitamin D and CRC risk was statistically significant only in men with calcium intake above 885 mg/d. However, the opposite was observed in the Nurses' Health Study (47). No interaction was detected between dietary calcium and circulating 25-hydroxyvitamin D level in the EPIC cohort (5). Several studies also investigated potential interactions between VDR polymorphisms and calcium and vitamin D intakes or status. An American study observed a significant 40% reduction in risk of rectal cancer for the SS (polyA) or BB (BsmI) VDR genotypes when calcium intake was low (70). The positive association between the ff genotype and CRC risk could be stronger among individuals with lower calcium intake (50). However, the opposite was observed in a large Scottish case-control study (19). Finnaly, in the EPIC study, Jenab et al. observed that the inverse CRC risk association of higher 25-hydroxyvitamin D was stronger at lower intakes of retinol (5). This interaction was not observed in the Health Professionals' Follow-up and the Nurses' Health Studies (47). No interaction between 25-hydroxyvitamin D and alcohol was detected in the EPIC study (5). To date, data are still insufficient to draw firm conclusions on gene-diet-vitamin D status interactions and CRC.

Since we did not have original data, we were not able to systematically take into consideration or meta-analyze the potential interactions between vitamin D and dietary, lifestyle, environmental and genetic effect modifiers.

Original aspects of our study included an updated meta-analysis of prospective studies on CRC risk and vitamin D intake and status, with dose-response analyses, which strengthens the plausibility of a causal association. We also used non-linear dose-response models for 25-hydroxyvitamin D data, in order to investigate the potential for a threshold effect. Finally, we conducted meta-analyses of all single-nucleotide VDR polymorphisms for which sufficient data was available. These complementary investigations allowed us to draw an overview of the relationship between vitamin D and CRC risk.

Limitations of our study should be considered. First, three publications were not included in the meta-analyses due to insufficient data. These publications suggested either no association of CRC risk with the main VDR polymorphisms studied (22) and with dietary vitamin D intake (41), or an inverse association with dietary vitamin D intake (40).

Second, it is possible that the observed relationships could be partly due to unmeasured or residual confounding. For instance, CRC risk was statistically significantly associated with dietary but not total vitamin D intake in dose-response analyses. This could be related to the fact that several medical conditions (among which some may be cancer precursors) may motivate the subjects (rightly or wrongly) to take supplements (71). In addition, there is compelling evidence in the literature that soy intake can influence the metabolism of vitamin D (72) and therefore may be a potential confounder. To our knowledge, none of the included study adjusted their analyses on soy or isoflavone intake or phytoestrogen supplement use. Besides, most studies on vitamin D intake could not control for sun exposure. This lack of data on sun exposure was compensated by the consideration of studies based on a biomarker of vitamin D status. However, the concentration of 25-hydroxyvitamin D in serum/plasma is considered as an accurate biomarker of vitamin D status (73), but a single cross-sectional measurement

(as done in all studies reviewed) does not take into account potential seasonal variations and could lead to non-differential classification bias. Nevertheless, most of the studies included in this meta-analysis adjusted for known confounding factors such as age, body mass index, smoking, alcohol, physical activity, red/processed meat intake, energy intake, and season of blood draw (for studies on vitamin D status). Beyond a potential confounding effect, season of blood collection may also interact with vitamin D status on the risk of CRC. In the Health professionals' follow-up study, the relationship between 25-hydroxyvitamin D and CRC risk was statistically significant for subjects whose blood collection occurred during the winter, but not during the summer (47). In the Nurses' Health Study, 25-hydroxyvitamin D was inversely associated with CRC risk only in areas with >335 langleys/day of UV light (36).

Next, the imperfections associated with published information may constitute limitations of the meta-analyses. Notably, some limitations are specific to studies based on dietary data collection. The associations estimated in our meta-analysis were weak. Measurement errors in the assessment of dietary/supplemental intake and uncertainty of information used from food composition tables are known to bias estimates. However, since we included only prospective studies, the measurement errors would most likely be non-differential. Besides, the prospective design of the included studies also minimized the possibility of recall or selection bias. Dietary changes after baseline may, however, attenuate associations between dietary intake of vitamin D and cancer risk, as studies generally considered only baseline intake.

Finally, in some analyses, our statistical power was limited when investigating associations with specific outcome subtypes (i.e., proximal and distal colon cancer) and/or specific exposures (i.e., supplemental vitamin D intake). Similarly, other single-nucleotide VDR polymorphisms such as tru91 or other variants (22, 52) have also been investigated in association with CRC risk, but to date, we were not able to perform meta-analyses on these variants due to insufficient data.

Experimental studies support a protective effect of vitamin D on CRC. Some animal studies indicated that vitamin D status may influence growth of intestinal tumors (74-77). Vitamin D status

387 modulates various genes in the colorectal mucosa that may influence cancer risk (69, 78). In humans,
388 vitamin D may induce differentiation and apoptosis (79, 80), both in colorectal adenoma or cancer cells
389 (81) and in the normal colorectal epithelium (82-84).
390
391 In conclusion, the quantitative summary of the existing evidence from prospective cohort studies
392 supports a modest although significant influence of vitamin D on colorectal carcinogenesis. The
393 conclusions are supported by analysis on vitamin D intake but also on a biomarker of vitamin D status
394 and on a VDR polymorphism. Available studies in vitamin D supplementation did not provide evidence
395 of a benefit beyond that observed for dietary intake of vitamin D. Randomized controlled trials may more
396 definitively establish a causal association, but the current data are sparse and inconclusive (85, 86) and
397 long follow-up time will be needed before a substantial number of CRC cases could be identified in
398 ongoing or future trials. So far, recommendations for CRC prevention should still mainly rely on the
399 results of prospective observational studies.
400 Given the potential benefits from vitamin D against CRC, further research should be a priority.
401 Beyond the protective effect on CRC risk suggested by this meta-analysis, vitamin D is implicated in fall
402 and fracture prevention and dental health, and may also reduce incident hypertension and cardiovascular
403 mortality and convey immune-modulatory and anti-inflammatory benefits (87). This underlines the public
404 health importance of reaching and maintaining an optimal vitamin D status at all life stages.

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Table 1. Summary relative risks of meta-analyses for the associations of colorectal, colon and rectum cancer with vitamin D intake and status (dose-response analyses) and vitamin D receptor polymorphisms

	Colorectal cancer			Colon cancer			Rectum cancer		
	Summary RR (95% CI)	n	I^2, $P_{heterogeneity}$	Summary RR (95% CI)	n	I^2, $P_{heterogeneity}$	Summary RR (95% CI)	n	I^2, $P_{heterogeneity}$
Vitamin D Intake (for an increase of 100 IU/day)									
Dietary vitamin D	0.95 (0.93-0.98)	10	11.0%, *P* =0.34	0.97 (0.92-1.02)	8	0.0%, *P* =0.66	0.91 (0.77-1.08)	6	58.9%, *P* =0.03
Included studies			*(4-7, 26, 42-45, 88)*			*(4, 5, 26-28, 42-44)*			*(4, 5, 26, 42-44)*
Supplemental vitamin D				0.93 (0.88-0.98)	2	0.0%, *P* =0.98			
Included studies						*(27, 28)*			
Total vitamin D	0.98 (0.95-1.01)	5	61.7%, *P* =0.034	0.93 (0.90-0.98)	4	49.5%, *P* =0.12	0.99 (0.94-1.04)	3	5.6%, *P* =0.35
Included studies			*(7, 26, 27, 30, 42, 45)*			*(26-28, 42)*			*(26, 30, 42)*
Biomarker of vitamin D status (for an increase of 100 IU/l)									
25-hydroxyvitamin D	0.96 (0.94-0.97)	6	0.0%, *P* =0.81	0.95 (0.92-0.995)	6	47.9%, *P* =0.09	0.95 (0.86-1.05)	5	66.7%, *P* =0.02
Included studies			*(5, 12, 46, 47, 89)*			*(5, 46, 47, 89, 90)*			*(5, 46, 47, 89)*
Vitamin D receptor polymorphisms									
FokI: fF vs. FF	1.03 (0.87-1.22)	10	71.3%, *P* <0.0001	1.04 (0.79-1.36)	4	70.9%, *P* =0.02	1.01 (0.82-1.23)	3	15.5%, *P* =0.31
Included studies			*(19, 21, 48-55)*			*(21, 50, 56, 57)*			*(21, 50, 51)*
ff vs. FF	0.98 (0.74-1.30)	10	80.8%, *P* <0.0001	1.26 (0.76-2.11)	4	85.7%, *P* <0.0001	1.03 (0.80-1.34)	3	13.0%, *P* =0.32
Included studies			*(19, 21, 48-55)*			*(21, 50, 56, 57)*			*(21, 50, 51)*
BsmI: Bb vs. bb	0.81 (0.64-1.02)	9	86.8%, *P* <0.0001	1.01 (0.89-1.14)	2	0.0%, *P* =0.96	0.97 (0.70-1.33)	3	55.8%, *P* =0.10
Included studies			*(19-21, 48, 49, 51, 52, 91, 92)*			*(21, 51)*			*(21, 51, 93)*
BB vs. bb	0.57 (0.36-0.89)	8	94.0%, *P* <0.0001	0.82 (0.66-1.02)	2	22.4%, *P* =0.26	0.95 (0.76-1.20)	3	0.0%, *P* =0.97
Included studies			*(19-21, 48, 51, 52, 91, 92)*			*(21, 51)*			*(21, 51, 93)*
TaqI: Tt vs. TT	1.00 (0.74-1.35)	5	45.9%, *P* =0.12	1.04 (0.78-1.39)	2	0.0%, *P* =0.75			
Included studies			*(20, 49, 52, 55, 58)*			*(57, 94)*			
tt vs. TT	1.34 (0.80-2.24)	4	64.7%, *P* =0.04	0.98 (0.36-2.66)	2	86.1%, *P* =0.007			
Included studies			*(20, 52, 55, 58)*			*(57, 94)*			
Cdx2: Cc vs. cc	1.09 (1.001-1.18)	4	0.0%, *P* =0.80	1.04 (0.91-1.20)	2	0.0%, *P* =0.90			
Included studies			*(19, 20, 52, 95)*			*(57, 95)*			
CC vs. cc	1.11 (0.94-1.32)	4	0.0%, *P* =0.98	1.43 (0.76-2.68)	2	52.1%, *P* =0.15			
Included studies			*(19, 20, 52, 95)*			*(57, 95)*			
PolyA: LS vs. LL	0.93 (0.82-1.06)	2	0.0%, *P* =0.62						
Included studies			*(70, 96, 97)*						
SS vs. LL	0.84 (0.66-1.06)	2	0.0%, *P* =0.35						
Included studies			*(70, 96, 97)*						
ApaI: Aa vs. aa	0.95 (0.80-1.13)	5	32.2%, *P* =0.21						
Included studies			*(19, 20, 49, 52, 58)*						
AA vs. aa	0.91 (0.67-1.23)	5	65.0%, *P* =0.02						
Included studies			*(19, 20, 49, 52, 58)*						

Note: n denotes the number of studies included.

Figure legends

Figure 1. Flow-chart of study selection for the association of vitamin D intake and status (prospective studies) and VDR polymorphisms with colorectal cancer (up until June 2010)

Figure 2. Dose-response meta-analyses on dietary and total (dietary + supplemental) vitamin D intake, circulating 25-hydroxyvitamin D and risk of colorectal, colon and rectal cancer

A. Dietary vitamin D (for an increase of 100 IU/d)

B. Total vitamin D (for an increase of 100 IU/d)

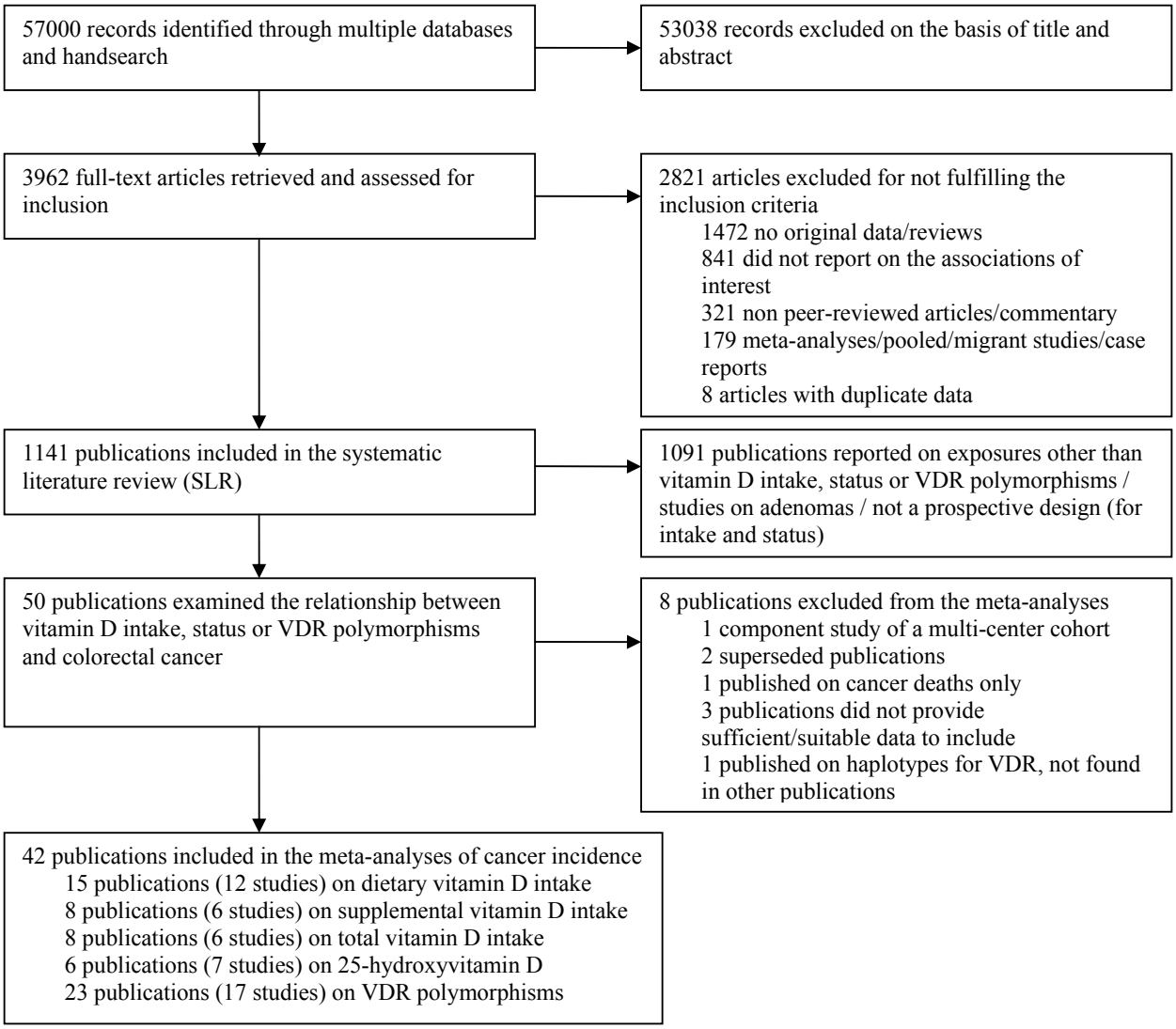
C. Circulating 25-hydroxyvitamin D (for an increase of 100 IU/l)

Figure 3. **Non-linear dose-response meta-analyses on circulating 25-hydroxyvitamin D and risk of colorectal cancer**

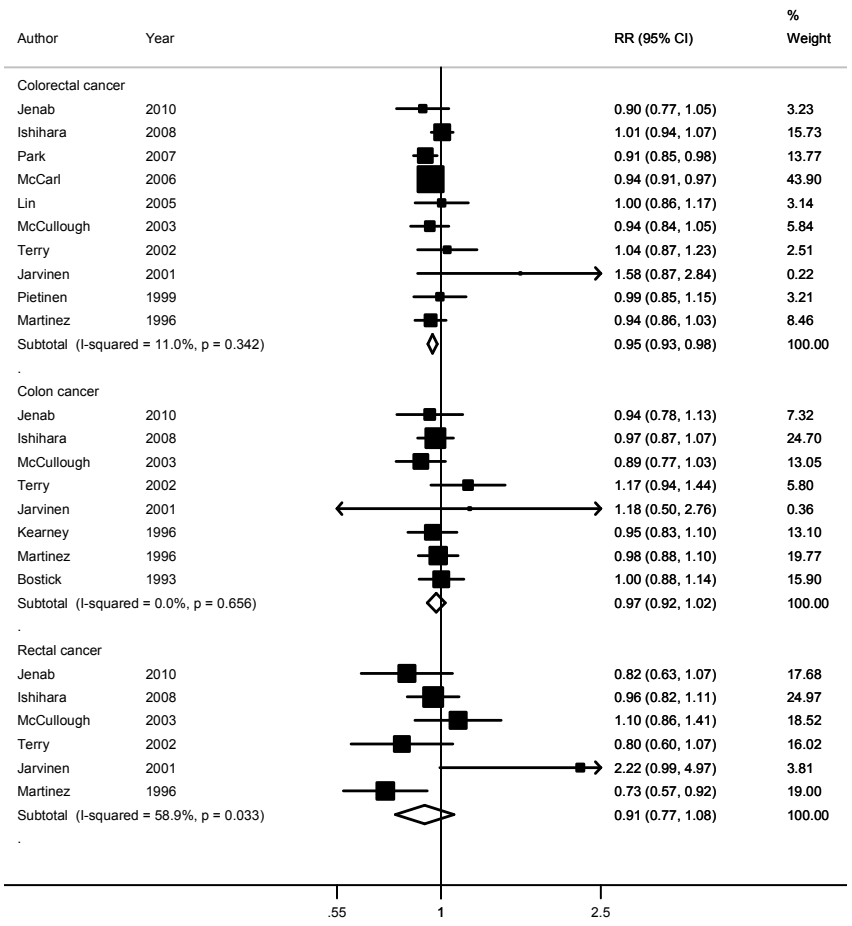
Figure 4. **Meta-analyses on BsmI vitamin D receptor polymorphism and risk of colorectal cancer**

A. Bb (heterozygous type) vs. bb (wild type)

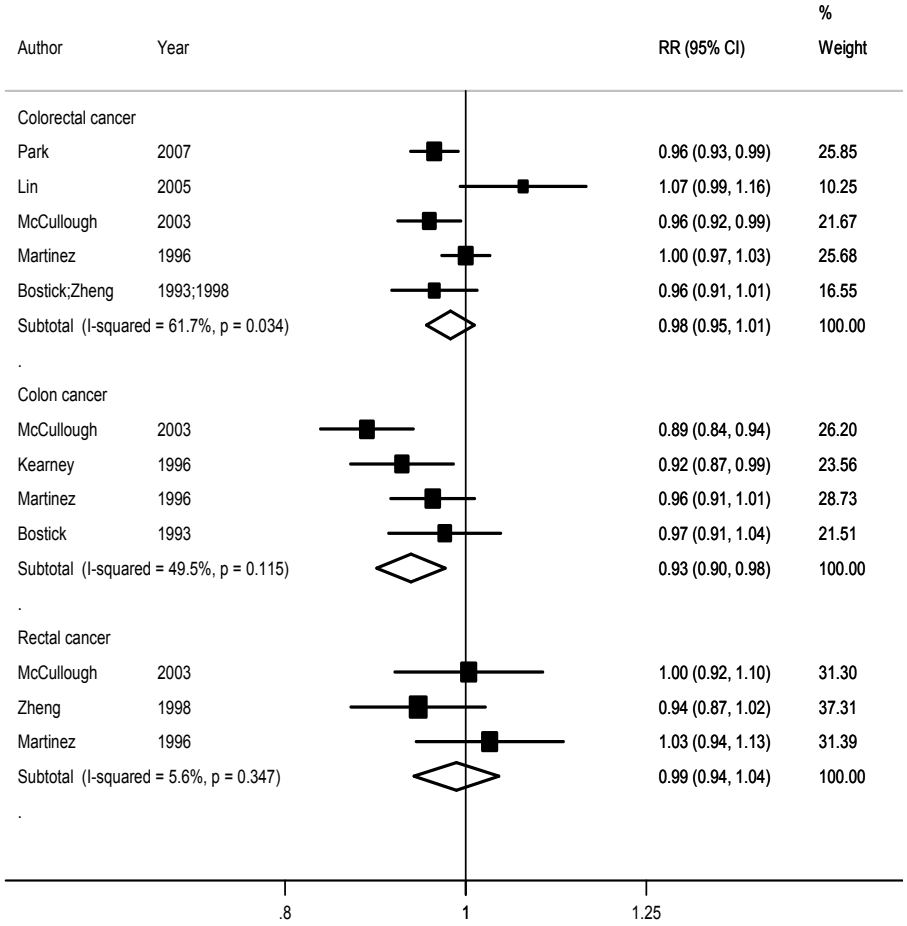
B. BB (homozygous mutant type) vs. bb (wild type)



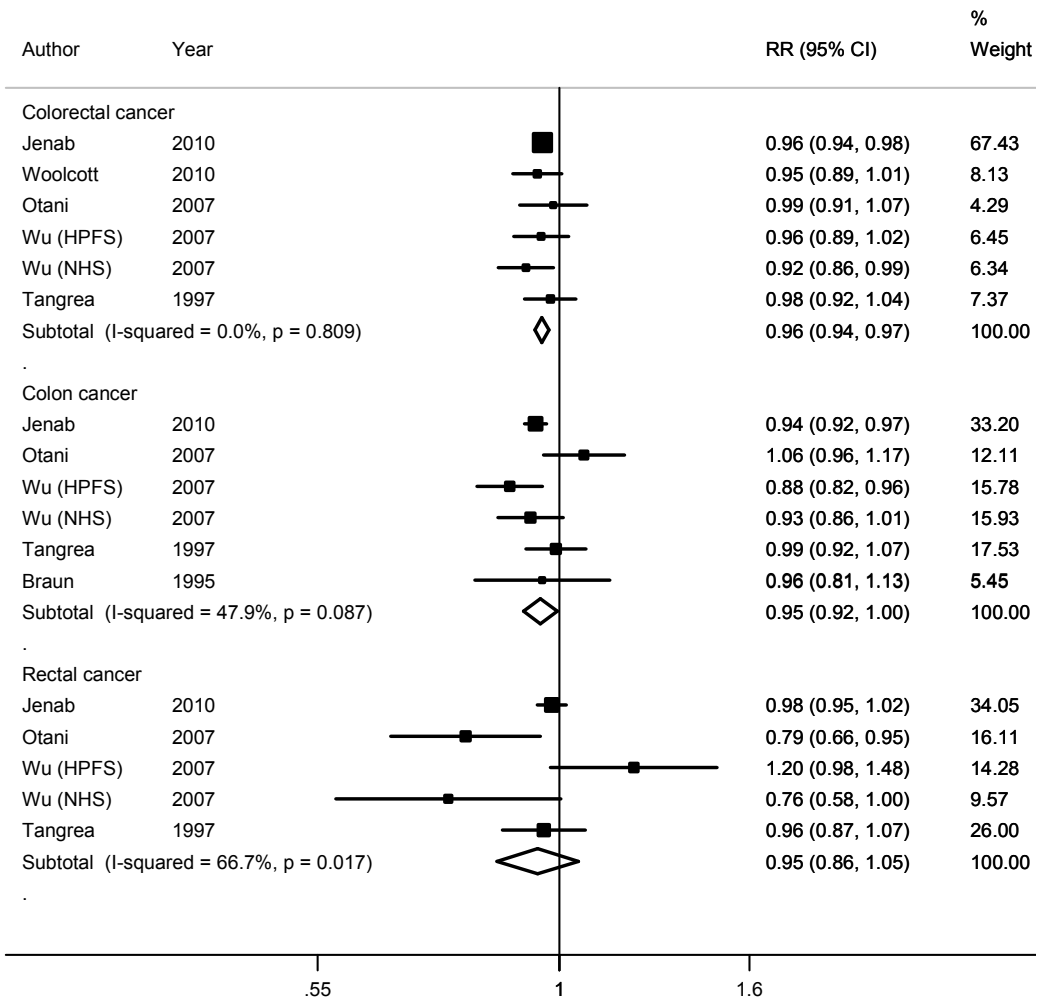
A.

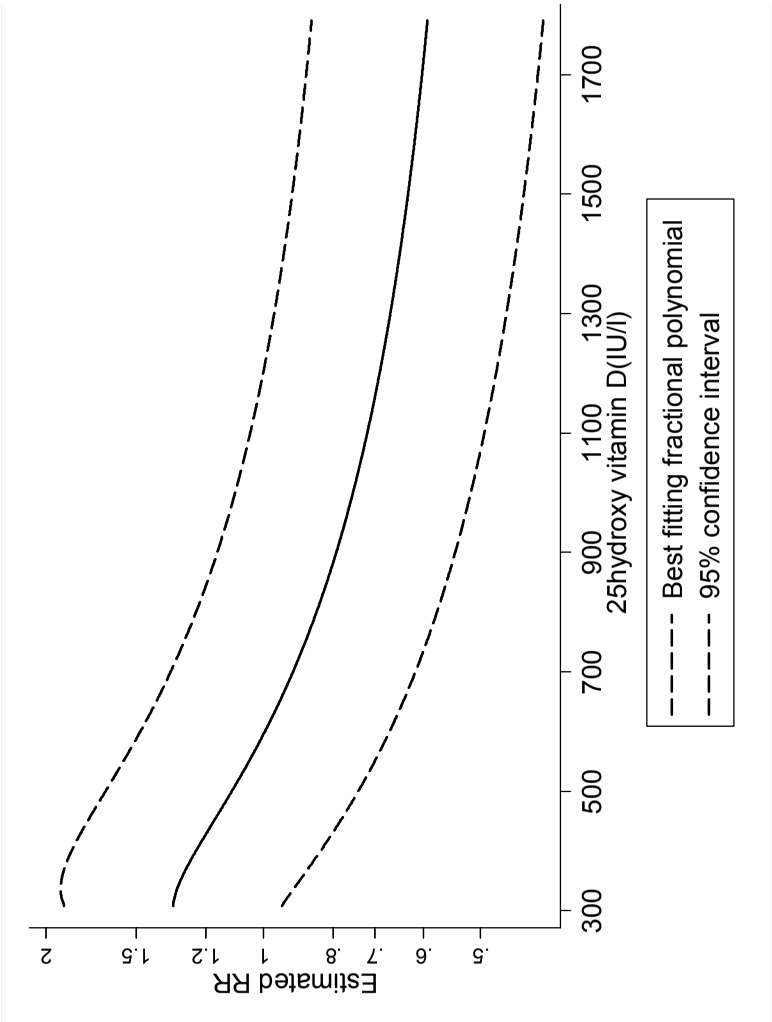


B.

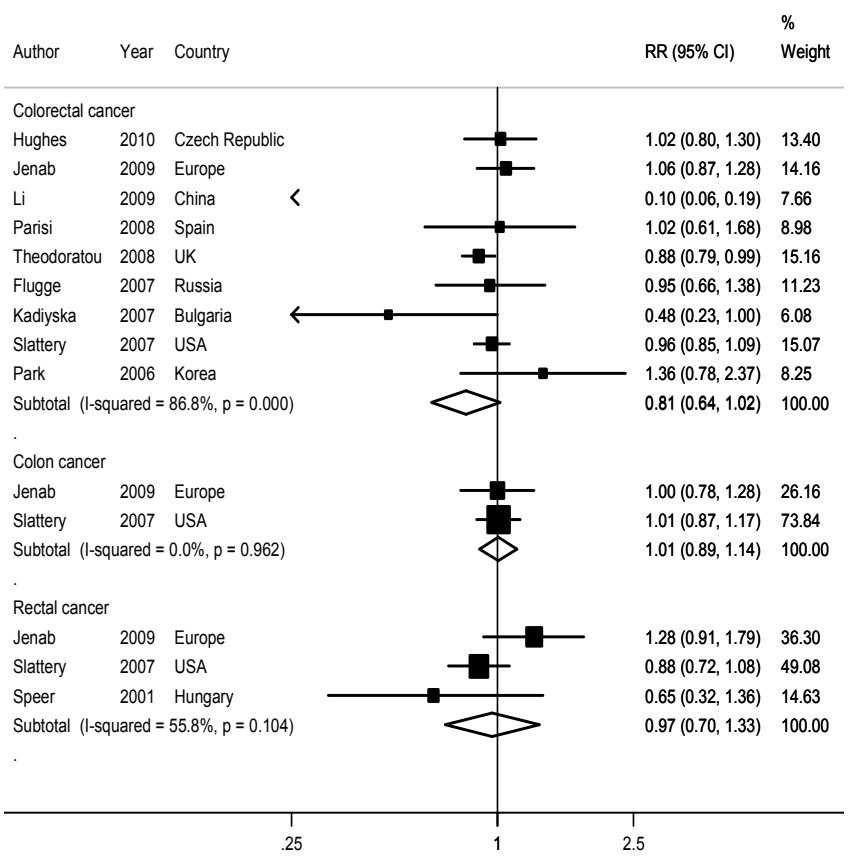


C.





A.



B.