**Circulating Vitamin D levels and colorectal cancer risk: a meta-analysis and systematic review of case-control and prospective cohort studies**

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# **MOOSE checklist**

From: Stroup DF, Berlin JA, Morton SC, et al (2000) Meta-analysis of observational studies in epidemiology: A proposal for reporting. JAMA 283:2008–2012. doi:10.1001/jama.283.15.2008.

|  |  |  |
| --- | --- | --- |
|  | Reported on page | Comments |
| **Reporting of background should include** | | |
| Problem definition | 4-5 |  |
| Hypothesis statement | 5 |  |
| Description of study outcome(s) | 8 |  |
| Type of exposure or intervention used | 6-7 |  |
| Type of study designs used | 6-7 |  |
| Study population | 6-7 |  |
| **Reporting of search strategy should include** | | |
| Qualifications of searchers (e.g. librarians and investigators) |  | The qualifications of searchers are reported in the protocol, which is free available at PROSPERO. |
| Search strategy, including time period used in the synthesis and key words | 6 and Supp. Table 1 |  |
| Effort to include all available studies, including contact with authors | 7 |  |
| Databases and registries searched | 6 |  |
| Search software used, name and version, including special features used (e.g. explosion) | 6 |  |
| Use of hand searching (e.g. reference lists of obtained articles) | 6-7 |  |
| List of citations located and those excluded, including justification |  | The PRISMA flow chart describes the process of the literature search process. The citation list of excluded articles is available upon request |
| Method of addressing articles published in languages other than English |  | Our search strategy did not identify any article published in languages other than English |
| Method of handling abstracts and unpublished studies | 7 |  |
| Description of any contact with authors | 7 | We did not contact any author for asking extra information |
| **Reporting of methods should include** | | |
| Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested | 8 |  |
| Rationale for the selection and coding of data (e.g. sound clinical principles or convenience) | 8-10 |  |
| Documentation of how data were classified and coded (e.g. multiple raters, blinding and interrater reliability) | 8-10 |  |
| Assessment of confounding (e.g. comparability of cases and controls in studies where appropriate) |  | We use the Study Quality Assessment of Case-Control Studies from the National Heart, Lung, and Blood institute and the Newcastle-Ottawa scale to rate the quality of the included studies. Both tools have items to evaluate confounding in the original articles. |
| Assessment of study quality, including blinding of quality assessors, stratification or regression on possible predictors of study results | 7-8 | The study quality of prospective cohort studies was assessed using the Newcastle-Ottawa scale. For case-control studies, we used The Study Quality Assessment of Case-Control Studies from the National Heart, Lung, and Blood Institute |
| Assessment of heterogeneity | 9-10 | Inter-study heterogeneity was assessed using the Cochran Q statistic and quantified using the I2 statistic, where I2>50% at PQ<0.10 was considered evidence of substantial heterogeneity |
| Description of statistical methods (e.g. complete description of fixed or random effects models, justification of whether the chosen models account for predictors of study results, dose-response models, or cumulative meta-analysis) in sufficient detail to be replicated | 9-10 |  |
| Provision of appropriate tables and graphics | Tables 1-2, figures 1-5 and supp. material |  |
| **Reporting of results should include** | | |
| Graphic summarizing individual study estimates and overall estimate | Figure 2-5 and supp. figures 1-3 and 6 |  |
| Table giving descriptive information for each study included | Table 1 and 2 |  |
| Results of sensitivity testing (e.g. subgroup analysis) | 16-19 and  Supp. tables 2 and 3 |  |
| Indication of statistical uncertainty of findings | 15, 19-20 |  |
| **Reporting of discussion should include** | | |
| Quantitative assessment of bias (e.g. publication bias) | 15-16 |  |
| Justification for exclusion (e.g. exclusion of non-English language citations) | 6-7 | Exclusion criteria are stated in material and methods section |
| Assessment of quality of included studies | 20 | Quality assessment is indicated in the Results’ section |
| **Reporting of conclusions should include** | | |
| Consideration of alternative explanations for observed results | 23-24 |  |
| Generalization of the conclusions (i.e. appropriate for the data presented and within the domain of the literature review) | 23-24 |  |
| Guidelines for future research | 24 |  |
| Disclosure of funding source | 25 |  |

# **Supplementary Table 1.** Search strategy

|  |
| --- |
| **PUBMED SEARCH**  (using R packages “pubmed.mineR” and “RISmed”) |
| '("colorectal neoplasms"[MeSh] OR  ("colorectal"[ALL] AND "neoplasms"[ALL]) OR  "colorectal neoplasms"[ALL] OR  ("colorectal"[ALL] AND "cancer"[ALL]) OR  "colorectal cancer"[ALL] OR  "colon cancer"[ALL] OR  "rectal cancer"[ALL] OR  "colon neoplasm"[ALL] OR  "rectal neoplasm"[ALL] OR  "colon polyp"[ALL] OR  "rectum polyp"[ALL] OR  "rectum adenocarcinoma"[ALL] OR  "colon adenocarcinoma"[ALL]) AND  ("vitamin d"[MeSh] OR  "vitamin d"[ALL] OR  "ergocalciferols"[MeSh] OR  "ergocalciferols"[ALL] OR  "25-hydroxyvitamin D"[ALL] OR  "vitamin D2"[ALL] OR  "vitamin D3"[ALL] OR  "cholecalciferol"[ALL])' |
| **COCHRANE SEARCH** |
| #1 MeSH descriptor: [Colorectal Neoplasms] explode all trees  #2 (colorectal neoplasms):ti,ab,kw  #3 (colorectal cancer):ti,ab,kw  #4 (colon cancer):ti,ab,kw  #5 (rectal cancer):ti,ab,kw  #6 (colon neoplasm):ti,ab,kw  #7 (rectal neoplasm):ti,ab,kw  #8 (colon polyp):ti,ab,kw  #9 (rectum polyp):ti,ab,kw  #10 (rectum adenocarcinoma):ti,ab,kw  #11 (colon adenocarcinoma):ti,ab,kw  #12 #1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11  #13 MeSH descriptor: [Hydroxycholecalciferols] explode all trees  #14 (vitamin d):ti,ab,kw  #15 (ergocalciferols):ti,ab,kw  #16 (25(OH)D):ti,ab,kw  #17 ("25-hydroxyvitamin-D"):ti,ab,kw  #18 (vitamin D2):ti,ab,kw  #19 (vitamin D3):ti,ab,kw  #20 (cholecalciferol):ti,ab,kw  #21 #13 OR #14 OR #15 OR #16 OR #17 OR #18 OR #19 OR #20  #22 #12 AND #21 |

# **Supplementary Table 2.** Influence analysis using the leave-one out approach for the meta-analyses assessing the association between circulating vitamin D levels (highest versus lowest categories) and the risk of colon, rectal and colorectal cancer.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Author** | **OR/HR (95% CI)** | **% change OR/HR** | **I2** | **Tau2** |
| **CCS – CRC – 25(OH)D – All subjects** | 0.61 (0.52; 0.71) | NA | 21 | 0.0125 |
| Omitting McCullough et al. 2018 | 0.58 (0.49; 0.68) | -4.95 | 11 | 0.0089 |
| Omitting Otani et al. 2007 [Women] | 0.59 (0.51; 0.68) | -2.53 | 11 | 0.0055 |
| Omitting Andersen et al. 2017 | 0.60 (0.51; 0.71) | -1.47 | 24 | 0.0155 |
| Omitting Ordóñez-Mena et al. 2015 [EPIC] | 0.60 (0.51; 0.70) | -1.29 | 24 | 0.0143 |
| Omitting Otani et al. 2007 [Men] | 0.60 (0.51; 0.71) | -0.46 | 27 | 0.0167 |
| Omitting Acikgoz et al. 2020 | 0.61 (0.52; 0.71) | 0.04 | 28 | 0.0170 |
| Omitting Woolcott et al. 2010 | 0.61 (0.52; 0.72) | 0.62 | 28 | 0.0185 |
| Omitting Weinstein et al. 2015 | 0.61 (0.52; 0.73) | 0.87 | 28 | 0.0191 |
| Omitting Jenab et al. 2010 | 0.61 (0.52; 0.73) | 0.98 | 28 | 0.0191 |
| Omitting Ying et al. 2015 | 0.62 (0.53; 0.72) | 2.03 | 22 | 0.0129 |
| Omitting Theodoratou et al. 2012 | 0.66 (0.58; 0.76) | 9.55 | 0 | 0 |
| **CCS – CRC – 25(OH)D – Men** | 0.78 (0.55; 1.11) | NA | 78\* | 0.1959 |
| Omitting Anic et al. 2014 | 0.69 (0.51; 0.93) | -11.85 | 63\* | 0.1008 |
| Omitting Weinstein et al. 2011 | 0.72 (0.50; 1.05) | -7.13 | 78\* | 0.1821 |
| Omitting McCullough et al. 2018 | 0.77 (0.50; 1.19) | -0.68 | 81\* | 0.2616 |
| Omitting Otani et al. 2007 | 0.79 (0.53; 1.16) | 0.88 | 81\* | 0.2180 |
| Omitting Hiraki et al. 2014 [PHS] | 0.80 (0.53; 1.20) | 2.32 | 81\* | 0.2286 |
| Omitting Hiraki et al. 2014 [HPFS] | 0.80 (0.53; 1.21) | 2.59 | 81\* | 0.2400 |
| Omitting Tangrea et al. 1997 | 0.81 (0.54; 1.20) | 3.43 | 81\* | 0.2190 |
| Omitting Theodoratou et al. 2012 ¥ | 0.88 (0.66; 1.18) | 12.69 | 55\* | 0.0813 |
| **CCS – CRC – 25(OH)D – Women** | 0.52 (0.41; 0.67) | NA | 39 | 0.0333 |
| Omitting McCullough et al. 2018 | 0.51 (0.35; 0.73) | -3.16 | 49⸸ | 0.0815 |
| Omitting Otani et al. 2007 | 0.51 (0.42; 0.61) | -3.05 | 12 | 0.0061 |
| Omitting Song et al. 2016 | 0.52 (0.38; 0.70) | -1.23 | 51⸸ | 0.0562 |
| Omitting Theodoratou et al. 2012 | 0.53 (0.38; 0.73) | 0.46 | 50⸸ | 0.0673 |
| Omitting Chandler et al. 2015 | 0.53 (0.40; 0.70) | 1.34 | 50⸸ | 0.0476 |
| Omitting Neuhouser et al. 2012 | 0.55 (0.47; 0.65) | 5.47 | 0 | 0 |
| **CCS – CC – 25(OH)D – Men** | 1.06 (0.62; 1.83) | NA | 70\* | 0.2684 |
| Omitting Weinstein et al. 2011 | 0.87 (0.53; 1.44) | -18.03 | 52 | 0.1374 |
| Omitting Lee et al. 2011 | 0.99 (0.49; 1.99) | -6.83 | 76\* | 0.3834 |
| Omitting Otani et al. 2007 | 1.03 (0.53; 2.01) | -2.85 | 77\* | 0.359 |
| Omitting Tangrea et al. 1997 | 1.14 (0.58; 2.23) | 6.95 | 76\* | 0.3522 |
| Omitting Wu et al. 2007 | 1.35 (0.88; 2.05) | 26.63 | 36 | 0.0657 |
| **CCS – RC – 25(OH)D – Men** | 0.73 (0.30; 1.79) | NA | 64\* | 0.6142 |
| Omitting Wu et al. 2007 | 0.53 (0.22; 1.30) | -26.85 | 55⸸ | 0.4294 |
| Omitting Weinstein et al. 2011 | 0.57 (0.16; 2.06) | -21.60 | 68\* | 1.1099 |
| Omitting Lee et al. 2011 | 0.81 (0.26; 2.45) | 10.57 | 68\* | 0.8105 |
| Omitting Tangrea et al. 1997 | 0.84 (0.29; 2.46) | 15.11 | 67\* | 0.7393 |
| Omitting Otani et al. 2007 | 0.91 (0.40; 2.08) | 25.21 | 59⸸ | 0.4090 |
| **PCS – CRC – 25(OH)D – All subjects** | 0.80 (0.66; 0.97) | NA | 0 | 0 |
| Omitting Ordóñez-Mena et al. 2015 [ESTHER] | 0.77 (0.63; 0.95) | -3.72 | 0 | 0 |
| Omitting Zhu et al. 2019 | 0.78 (0.64; 0.96) | -2.60 | 0 | 0 |
| Omitting Skaaby et al. 2014 | 0.80 (0.65; 0.99) | -0.35 | 0 | 0 |
| Omitting Ordóñez-Mena et al. 2015 [Tromsø] | 0.81 (0.66; 0.99) | 0.76 | 0 | 0 |
| Omitting Ordóñez-Mena et al. 2013 | 0.81 (0.66; 1.00) | 1.11 | 0 | 0 |
| Omitting Heath et al. 2020 | 0.86 (0.68; 1.09) | 6.71 | 0 | 0 |

Data show the leave-one out approach for the different meta-analyses with more than 4 study comparisons from independent studies. Red font denotes influential studies (i.e., study changing the evidence of heterogeneity or the magnitude by more than 20%, the significance and/or direction of the association). Meta-analyses were performed using generic inverse-variance random effects models. Legend for studies: ¥, reported as an outlier (i.e., study’s original confidence interval does not overlap with the confidence interval of the pooled effect). **Legend for I2 column:** \*, P-value ≤0.05; ⸸, P-value >0.05 and <0.10. **Abbreviations: CC**, colon cancer; **CCS**, case-control studies; **CI**, confidence interval; **CRC**, colorectal cancer; **NA**, not applicable; **PCS**, prospective cohort studies; **RC**, rectal cancer; **Vit**, vitamin.

# **Supplementary Table 3.** Subgroup analyses considering the methodological procedure and source of sample for circulating vitamin D determination.

|  |  |  |  |
| --- | --- | --- | --- |
| **Study** | **OR/HR (95% CI)** | **I2** | **Relevant change versus overall** |
| **CCS – CRC – 25(OH)D – All subjects** | 0.61 [0.52; 0.71] | 21 | NA |
| **METHOD** (Subgroup differences: *P*=0.08) |  |  |  |
| CLIA: 4 studies | 0.67 [0.57; 0.78] | 0 | No |
| **SAMPLE** (Subgroup differences: *P*=0.51) |  |  |  |
| Plasma: 5 studies | 0.56 [0.41; 0.75] | 37.9 | No |
| Serum: 4 studies | 0.63 [0.47; 0.85] | 0 | No |
| Circulating: 2 studies | 0.69 [0.57; 0.82] | 0 | No |
| **CCS – CRC – 25(OH)D – Men** | 0.78 [0.55; 1.11] | 78\* | NA |
| **METHOD** (Subgroup differences: *P*=0.004) |  |  |  |
| CLIA: 3 studies | 1.20 [0.75; 1.89] | 66.3\* | No |
| RISA: 2 studies | 0.67 [0.47; 0.95] | 0 | Yes |
| **SAMPLE** (Subgroup differences: *P*=0.07) |  |  |  |
| Plasma: 4 studies | 0.56 [0.41; 0.77] | 42.2 | Yes |
| Serum: 3 studies | 1.14 [0.63; 2.05] | 67.7\* | No |
| **CCS – CRC – 25(OH)D – Women** | 0.52 [0.41; 0.67] | 39 | NA |
| **METHOD** (Subgroup differences: *P*=0.24) |  |  |  |
| CLIA: 2 studies | 0.39 [0.16; 0.92] | 77\* | No |
| RIA: 2 studies | 0.51 [0.35; 0.75] | 0 | No |
| **SAMPLE** (Subgroup differences: *P*=0.11) |  |  |  |
| Plasma: 4 studies | 0.56 [0.42; 0.75] | 19.6 | No |
| **CCS – CC – 25(OH)D – Men** | 1.06 [0.62; 1.83] | 70\* | NA |
| **METHOD** (Subgroup differences: *P*=0.02) |  |  |  |
| RIA: 2 studies | 0.60 [0.35; 1.03] | 22.7 | No |
| **SAMPLE** (Subgroup differences: *P*=0.52) |  |  |  |
| Plasma: 3 studies | 0.90 [0.44; 1.84] | 67.6\* | No |
| Serum: 2 studies | 1.33 [0.51; 3.43] | 78\* | No |
| **CCS – RC – 25(OH)D – Men** | 0.73 [0.30; 1.79] | 64\* | NA |
| **METHOD** (Subgroup differences: *P*=0.13) |  |  |  |
| RIA: 2 studies | 1.13 [0.14; 8.96] | 81.2\* | No |
| **SAMPLE** (Subgroup differences: *P*=0.82) |  |  |  |
| Plasma: 3 studies | 0.61 [0.09; 3.98] | 76.5\* | No |
| Serum: 2 studies | 0.78 [0.28; 2.18] | 58.7\* | No |
| **PCS – CRC – 25(OH)D – All subjects** | 0.80 [0.66; 0.97] | 0 | NA |
| **METHOD** (Subgroup differences: *P*=0.93) |  |  |  |
| Immunosssays: 2 studies | 0.89 [0.61; 1.32] | 0 | Yes |
| LC-MS/MS: 2 studies | 0.77 [0.58; 1.02] | 0 | Yes |
| **SAMPLE** (Subgroup differences: *P*=0.36) |  |  |  |
| Serum: 5 studies | 0.86 [0.68; 1.09] | 0 | Yes |

Only subgroups with 2 or more studies are included. Note that the term “circulating” means that sample source was not described in their methods section. **Abbreviations: CLIA,** competitive chemiluminescence immunoassay; **CI**, confidence interval; **HR**, hazard ratio; **I2**, heterogeneity; **LC-MS/MS**, liquid chromatography-mass spectrometry; **NA**, not applicable**; OR**, odds ratio; **RIA**, radioimmunoassay; **RISA**, radioimmunosorbent assay**. \***, P<0.05.

**A) Case-control studies – Free 25(OH)D - All subjects**



**B) Case-control studies – 25(OH)D – Women**

Gráfico, Gráfico de cajas y bigotes

Descripción generada automáticamente

**C) Case-control studies – 25(OH)D – Men**

**Gráfico, Gráfico de cajas y bigotes

Descripción generada automáticamente**

**D) Prospective cohort studies - 25(OH)D – MenGráfico, Gráfico de cajas y bigotes

Descripción generada automáticamente**

**E) Case-control studies – 1,25(OH)2D - Men**

# **Supplementary Figure 1.** Forest plot for the association between circulating vitamin D levels (highest versus lowest categories) and risk of colorectal cancer (case-control and prospective studies). Meta-analyses were constructed using generic inverse-variance fixed-effects model (for meta-analysis with less than 5 studies) or random-effects model (for meta-analysis with 5 or more studies). **Abbreviations: CI**, confidence interval; **OR**, odds ratio.

**A) Case-control studies - 25(OH)D – All subjects**

**Gráfico de cajas y bigotes

Descripción generada automáticamente**

**B) Case-control studies - 25(OH)D – Men**

**Gráfico, Gráfico de cajas y bigotes

Descripción generada automáticamente**

**C) Case-control studies - 25(OH)D – Women**

**Gráfico, Gráfico de cajas y bigotes

Descripción generada automáticamente**

**D) Prospective cohort studies - 25(OH)D – All subjects**

**Gráfico, Gráfico de cajas y bigotes

Descripción generada automáticamente**

**E) Case-control studies – 1,25(OH)2D - Men**

****

# **Supplementary Figure 2.** Forest plot for the association between circulating vitamin D levels (highest versus lowest categories) and risk of colon cancer (case-control and prospective studies). Meta-analyses were constructed using generic inverse-variance fixed-effects model (for meta-analysis with less than 5 studies) or random-effects model (for meta-analysis with 5 or more studies). **Abbreviations: CI**, confidence interval; **HR**, hazard ratio; **OR**, odds ratio.

**A) Case-control studies – 25(OH)D – All subjects**

**Un conjunto de letras negras en un fondo blanco

Descripción generada automáticamente con confianza media**

**B) Case-control studies - 25(OH)D – Men**

**Diagrama

Descripción generada automáticamente con confianza media**

**C) Case-control studies - 25(OH)D - Women**

**Gráfico, Gráfico de cajas y bigotes

Descripción generada automáticamente**

**D) Case-control studies – 1,25(OH)2D - Men**

****

# **Supplementary Figure 3.** Forest plot for the association between circulating vitamin D levels (highest versus lowest categories) and risk of rectal cancer (case-control studies). Meta-analyses were constructed using generic inverse-variance fixed-effects model (for meta-analysis with less than 5 studies) or random-effects model (for meta-analysis with 5 or more studies). **Abbreviations: CI**, confidence interval; **OR**, odds ratio.

Gráfico

Descripción generada automáticamente

# **Supplementary Figure 4.** Funnel plot for detecting publication bias in the meta-analysis of case-control studies assessing the association between 25(OH)D and colorectal cancer in all the subjects. This contour-enhanced funnel plot shows the standard error and odds ratio for each of the studies of the meta-analysis. The different contour colors indicate the significance level (*see* legend in the plot) into which the effects size of each study falls. Egger’s test for funnel plot asymmetry was not significant (P=0.385). **Legend:** (**a**) Otani *et al.* 2007 [Men]; (**b**) Otani *et al.* 2007 [Women]; (**c**) Jenab *et al.* 2010; (**d**) Woolcott *et al.* 2010; (**e**) Theodoratou *et al.* 2012; (**f**) Ordóñez-Mena *et al.* 2015 [EPIC]; (**g**) Weinstein *et al.* 2015; (**h**) Ying *et al.* 2015; (**i**) Andersen *et al.* 2017; (**j**) McCullough *et al.* 2018; (**k**) Acikgoz *et al.* 2020. **Note that studies:** c and g overlap in the plot.

|  |  |
| --- | --- |
| **A)** | **B)** |
| **C)** | **D)** |
| **E)** | **F)** |

# **Supplementary Figure 5.** Graphic display of heterogeneity (GOSH) plot analyses for the different meta-analyses of circulating vitamin D levels. All the iterative meta-analyses (2studies – 1 individual analyses) were constructed using generic inverse-variance random-effects model. Plots are for: **A)** CCS – CRC – 25(OH)D – All subjects (11 studies, thus including 2047 possible subsets); **B)** CCS – CRC – 25(OH)D – Men (8 studies, thus including 255 possible subsets; please *see* **Supplementary Figure 5** for more details); **C)** CCS – CRC – 25(OH)D – Women (6 studies, thus including 63 possible subsets); **D)** CCS – CC – 25(OH)D – Men (5 studies, thus including 31 possible subsets); **E)** CCS – RC – 25(OH)D – Men (5 studies, thus including 31 possible subsets); **F)** PC – CRC – 25(OH)D – All (6 studies, thus including 63 possible subsets). **Abbreviations: CC**, colon cancer; **CCS**, case-control studies; **CRC**, colorectal cancer; **GOSH,** graphic display of heterogeneity; **PC**, prospective cohort; **RC**, rectal cancer.

|  |  |
| --- | --- |
| **A)** | **B)** |

# **Supplementary Figure 6.** Graphic display of heterogeneity (GOSH) plot analyses for the case-control meta-analysis evaluating colorectal cancer and circulating vitamin D levels in men. All the iterative meta-analyses (2studies – 1 individual analyses) were constructed using generic inverse-variance random-effects model. Plots are for: CCS – CRC – 25(OH)D – Men (8 studies, thus including 255 possible subsets), where b**lue** was used for plotting results considering iterative combinations excluding the influence studies/outliers (Theodoratou *et al.* 2012 in **A)**; and, Anic *et al.* 2014 in **B)**), whereas **red** color is used to plot results including each respective outlier. **Abbreviations: GOSH,** graphic display of heterogeneity.