Scientific Advisory Committee on Nutrition

Fortifying food and drink with vitamin D: annexes

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Annex 1: evidence sources for countries in scoping review

For full citations, see the references section of the main report.

Australia

Reference	Document type
Australian Government (2019)	Public health publication
Food Standards Australia New Zealand	Legislation document
Jayaratne and others (2013)	Journal article
Dunlop and others (2022)	Journal article

Canada

Reference	Document type
Government of Canada (2022a)	Legislation document
Government of Canada (2022b)	Legislation document
Calvo and others (2004)	Journal article
Government of Canada (2022c)	Legislation document
Calvo and others (2013)	Journal article
Langlois and others (2010)	Journal article
Statistics Canada (2015)	Public health publication

Sweden

Reference	Document type
Itkonen and others (2021)	Journal article
The Swedish Food Agency (2007)	Legislation document
The Swedish Food Agency (2018)	Legislation document
Nälsén and others (2020)	Journal article
Summerhays and others (2020)	Journal article

Finland

Reference	Document type
Lamberg-Allardt and others (2003)	Journal article
Ministry of Trade and Industry of Finland (2002)	Legislation document
Tylavsky and others (2006)	Journal article
Finnish National Nutrition Council (2010)	Public health publication
Lips P and others (2019)	Journal article
Pilz S and others (2018)	Journal article
Jääskeläinen and others (2017)	Journal article

Norway

Reference	Document type
Norwegian National Nutrition Council (2018)	Public health publication

Reference	Document type
Itkonen and others (2021)	Journal article
Oberg and others (2014)	Journal article

USA

Reference	Document
Calvo and others (2004)	Journal article
Food and Drug Administration (2016)	Legislation document
Food and Drug Administration (2022)	Legislation document
Fulgoni and others (2011)	Journal article
Moore and others (2014)	Journal article
Schleicher and others (2016)	Journal article
Calvo and others (2004)	Journal article
Calvo and others (2013)	Journal article
US Department of Agriculture (2022)	Public health publication

Annex 2: eligibility criteria

The following table shows the eligibility criteria for studies published after Balachandar and others (2021).

Criteria	Include	Exclude
Population	Studies involving apparently healthy individuals in all age groups	Studies explicitly intervening in patients with acute or chronic: cardiovascular disorders, liver disorders, kidney disorders, neuropsychiatric disorders, HIV or cancer
Intervention or exposure / comparator	Studies comparing ergocalciferol (vitamin D2) AND cholecalciferol (vitamin D3) by conventional supplementation or food fortification Include studies comparing: - any dosage - any route of administration - minimum 2 weeks of intervention For studies assessing outcome measures at multiple time points, the last measurement within 2 weeks of discontinuing treatment Include trials employing simultaneous co-interventions like health education, calcium intake and sunshine exposure if the only difference between intervention and comparison arms pertains to ergocalciferol and cholecalciferol	Any other intervention or control
Outcome	Serum concentration: total 25(OH)D or free 25(OH)D total 25(OH)D2 or free 25(OH)D2 total 25(OH)D3 or free 25(OH)D3 parathormone or parathyroid hormone (PTH)	Not applicable

Criteria	Include	Exclude
	25(OH)2D2 25(OH)2D3 total 1, 25(OH)2D	
Study design	Individual or cluster RCTs Individual or cluster non-RCTs with a concurrent comparison group, preferably with adjustment for baseline characteristics and confounders Controlled before-after (CBA) studies where allocation to the different comparison groups is not made by the investigators. The intervention group and control group should have been evaluated for the outcomes of interest at baseline and at the end of the study period Initial phase of crossover trials (pre-crossover period)	All other study designs: Systematic and non-systematic reviews Meta-analysis Pooled analysis Rapid reviews Cohorts Observational studies Modelling studies Laboratory studies Animal studies Preclinical studies In vitro studies
Literature type	Articles from peer reviewed journals (including preprints)	Abstract only Commentaries/opinion pieces Dissertations; conference proceedings; magazine articles; books, book chapters Information from websites Protocols
Language	English language only	Non-English language
Date	Articles published from 1 June 2021 to present	Articles published before 1 June 2021

Annex 3: search strategies

The tables below show the search strategies for different databases for studies published after Balachandar and others (2021).

Embase

Number	Searches	Results
1	exp *vitamin D/	72,407
2	("vitamin D2" and "vitamin D3").tw,kf.	709
3	(ergocalciferol and cholecalciferol).tw,kf.	493
4	1 or 2 or 3	72,611
5	blood level/	119,607
6	(("25 hydroxyvitamin D" or "25 OHD" or "25 OH D" or "IU") and (plasma or serum or "blood level*")).tw,kf.	50,927
7	5 or 6	167,742
8	4 and 7	14,134
9	limit 8 to randomized controlled trial	1,881
10	limit 9 to dc=20210601-20220811	198

MEDLINE (R) ALL

Number	Searches	Results
1	exp *Vitamin D/	44,713
2	("vitamin D2" and "vitamin D3").tw,kf.	446
3	(ergocalciferol and cholecalciferol).tw,kf.	323
4	1 or 2 or 3	44,853
5	(("25 hydroxyvitamin D" or "25 OHD" or "25 OH D" or "IU") and (plasma or serum or "blood level*")).tw,kf.	30,423
6	4 and 5	7,551
7	limit 6 to randomized controlled trial	1,335
8	limit 7 to dt=20210601-20220811	51

Scopus

The following search terms were used:

(((TITLE-ABS-KEY (ergocalciferol AND cholecalciferol)) OR (TITLE-ABS-KEY ("vitamin D2" AND "vitamin D3"))) AND (TITLE-ABS-KEY (("25 hydroxyvitamin D" OR "25 OHD" OR "25 OH D" OR "IU") AND (plasma OR serum OR "blood level*")))) AND (TITLE-ABS-KEY ("randomi?ed controlled trial" OR rct)) AND (LIMIT-TO (PUBYEAR , 2022) OR LIMIT-TO (PUBYEAR , 2021)) = 16

Annex 4: studies excluded after full text assessment

Intervention out of scope

The studies excluded after full text assessments because the intervention was out of scope were:

- Cereijo and others (2022)
- Galyean (2022)
- Olsen (2022)

Study design out of scope

The study excluded after a full text assessment because the study design was out of scope was Durrant (2022).

Annex 5: AMSTAR 2 assessment of the Balachandar systematic review

Domains	Yes or no
Did the research questions and inclusion criteria for the review include the components of PICO?	Yes
Did the report of the review contain an explicit statement that the review methods were established prior to the conduct of the review and did the report justify any significant deviations from the protocol?	Yes
Did the review authors explain their selection of the study designs for inclusion in the review?	Yes
Did the review authors use a comprehensive literature search strategy?	Partial yes
Did the review authors perform study selection in duplicate?	No
Did the review authors perform data extraction in duplicate?	No
Did the review authors provide a list of excluded studies and justify the exclusions?	No
Did the review authors describe the included studies in adequate detail?	Yes
Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included in the review?	Yes
Did the review authors report on the sources of funding for the studies included in the review?	No
If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?	Yes
If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?	No
Did the review authors account for RoB in individual studies when interpreting or discussing the results of the review?	No
Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?	Yes

Domains	Yes or no
If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?	Yes
Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?	Yes
Overall confidence	Low

Annex 6: vitamin D fortification policies and their impact

The following tables summarise the countries with mandatory and voluntary vitamin D fortification policies and their impact on intakes and status. For more detailed information, see section 4 in the main report.

Table A6.1: countries with mandatory vitamin D fortification policies

Country	Products fortified and fortification level	D2 or D3	Impact on intakes and status
Australia	Table edible oil, spread and table margarine: no less than 55µg (2,200 IU) per kg	Not specified	No trend data or studies identified that assessed impact of fortification.
Canada	Milk: 2µg (80 IU) per 100ml Margarine: 26µg (1,040 IU) per 100g	D2 or D3	No trend data or studies identified that assessed impact of fortification. Canadian Health Measures Survey (CHMS) (2007-9): consumption of at least one serving per day fortified milk associated with increments in plasma 25(OH)D concentrations of at least 6nmol per litre (Calvo and Whiting, 2013; Langlois and others, 2010). CHMS (2009-2011): individuals consuming milk once or more per day had a higher average plasma 25(OH)D concentration (68nmol per litre) than those who consumed milk less than once per day (59nmol per litre). Of those individuals who consumed milk once or more per day, 75% had plasma 25(OH)D concentrations above 50nmol per litre compared with 60% of those who had milk less than once per day (Statistics Canada, 2015).
Sweden	Less than 3% fat milk, between 0.95	Not specified	Time trends data between 1986 and 2014 (the Northern

Country	Products fortified and fortification level	D2 or D3	Impact on intakes and status
	and 1.10µg (38 to 44 IU) per 100g Less than 3% fermented milk products, 0.75 to 1.10µg (30 to 44 IU) per 100g Margarine, fat spreads and fluid margarine: 19.5 to 21.0 µg (780 to 840 IU) per 100g		Sweden MONICA Study) reported no clear upward or downward trend in serum 25(OH)D concentrations (Summerhays and others, 2020).

Table A6.2: countries with voluntary vitamin D fortification policies

Country	Products fortified and fortification level	D2 or D3	Impact on intakes and status
Finland	Fluid milk and yogurts: 1µg (40 IU) per 100g Fat spreads: 20 µg (800 IU) per 100g	D3 (D3 used in 2003; it is assumed that it was also used in 2010)	Finnish Health Survey in 2000 (before fortification) and 2011 (n=4051) (after fortification) (Jääskeläinen and others, 2017): In 2011, daily mean vitamin D intake from diet alone almost twice as high (men, 14µg or 560 IU; women, 12µg or 480 IU) than in 2,000 (men and women, 7µg or 280 IU). 74% of men and 58% of women achieved recommendation for vitamin D intake (10µg or 400 IU per day) from diet alone. Prevalence of serum 25(OH)D below 30nmol per litre decreased from 12% in 2000 to less than 1% in 2011. Mean serum 25(OH)D concentrations increased by 17.8nmol per litre, from 47.6nmol per litre in 2000 to 65.4nmol per litre in

Country	Products fortified and fortification level	D2 or D3	Impact on intakes and status
			2011.
Norway	Butter and margarine: up to 10 µg (400 IU) per 100g Milk (low fat): up to 0.4 µg (16 IU) per 100g	Not specified	No trend data or studies identified that assessed impact of fortification. Data from Tromsø Study (2010 to 2011) reported serum 25(OH)D concentrations in boys (age, 15-18 years) were significantly associated with consumption of vitamin D-fortified milk (Oberg and others, 2014).
USA	Milk: up to 2.1µg (84 IU) per 100g Plant-based beverage alternatives to milk: up to 2.1µg (84 IU) per 100g Plant-based yogurt alternatives: up to 2.23µg (89 IU) per 100g Breakfast cereals: up to 8.75µg (350 IU) per 100g Margarine: up to 8.23µg (329 IU) per 100g Yogurt: 2.23µg (89 IU) per 100g	Milk, breakfast cereals, margarines: D2 or D3 Plant based beverages and yogurt alternatives: D2	NHANES 2003 to 2006: mean vitamin D intake from food sources, was 4.9µg (196 IU) per day; natural food sources contributed 40.8% (2µg or 80 IU per day) and foods fortified with vitamin D contributed 59.2% (2.9µg or 116 IU per day) (Fulgoni and others, 2011). NHANES 2007 to 2010: fortified milk and milk products provided largest contribution (43.7%) to dietary vitamin D intakes of adults (Moore and others, 2014). NHANES 2007 to 2008: serum 25(OH)D concentrations highest in White Americans, then Mexican Americans, lowest in Black Americans. Fluid milk and ready to eat cereals major contributors to vitamin D intakes but consumption lowest for Black American adults (Calvo and others, 2004).

Annex 7: characteristics of daily dosing studies in the Balachandar systematic review

Table A7.1: studies that administered vitamins D2 and D3 at daily doses less than or equal to 25µg (1,000 IU) per day

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
Fisk and others (2012) (UK) Participants (n=40): Men (n=17) and women (n=23) (age, 18 to 65 years) Objective: to evaluate effect on serum 25(OH)D metabolites of 5 and 10 µg per day of D2 and D3 provided in a malted milk drink Study design: Double blind, controlled Conducted in winter Study power: n=8 per group required for 90% power (α=.01) to detect 10 nmol per litre change in 25(OH)D Funding: BBSRC and GlaxoSmithKline	Dose: 5 (200) and 10 (400) Groups: Placebo (n=8) 5 µg D2 (n=8) 5 µg D3 (n=8) 10 µg D2 (n=8) 10 µg D3(n=8) Duration: 4 weeks Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: LCMS	No difference between D2 and D3. Both groups significantly different from placebo	D2 did not affect 25(OH)D3. (D3 did not affect 25(OH)D2)	Both vitamin D2 and D3 fortified drinks resulted in dose-dependent increases ($p<0.001$) in their respective 25(OH) metabolites that did not significantly differ in size. Study indicates equivalent bioavailability of vitamin D2 and D3 in a malted milk drink. Supplementation with D2 did not influence serum concentrations of 25(OH)D3
Nimitphong and others (2013) (Thailand) Participants (n=39): Men (n=7) and	Dose: 10 (400) Groups: D2 (n=20)	No difference between D2 and D3	Caused significant decrease of 25(OH)D3	Vitamin D3 supplementation increased 25(OH)D3 significantly ($p<0.001$).

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
women (n=32) (age: 15 to 70 years) Objective: to investigate changes of total 25(OH)D, 25(OH)D3 and 25(OH)D2 concentrations in a Thai cohort, according to type of vitamin D supplement (D2 or D3) and vitamin D binding protein (DBP) genotype Study design: Unblinded, uncontrolled Conducted in winter Study power: not reported Funding: Faculty Research Fund, Ramathibodi Hospital	D3 (n=19) Duration: 3 months Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: LCMS MS	DBP genotype: CC (group 1) versus CA + AA (group 2) D2 intervention: No difference between 2 groups D3 intervention: Increase in 25(OH)D significantly lower in group 2	(p<0.001)	Vitamin D2 caused significant increase in 25(OH)D2 (p<0.001) together with a decrease of 25(OH)D3 (p<0.001). Vitamin D3 tended to increase total 25(OH)D more when compared with same dose of vitamin D2 (p=0.08). Underlying basis for this appears to be concurrent decrease in 25(OH)D3 after D2 supplementation. Genetic variation in DBP influences responsiveness to vitamin D3 but not D2.
Tripkovic and others (2017) (UK) Participants (n=270): Women (age: 20 to 64 years) Objective: to investigate whether vitamin D2 or D3 fortified in juice or	Dose: 15 (600) Groups: Placebo (n=65) D2 juice (n=67) D2 biscuit (n=66) D3 juice (n=70)	D3 more effective than D2	D2 group (D2 biscuit + D2 juice) decreased 25(OH)D3. D3 juice group	Vitamin D3 more effective than vitamin D2 in increasing serum 25(OH)D in the wintertime However, both forms

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
food (15 µg per day) was effective in increasing serum total 25(OH)D and to compare their respective efficacy Study design: Double blind, controlled Conducted in winter Study power: n=320 required for 90% power to detect: 0.6 SD (white women) and 1.1 SD (South Asian women) in 25(OH)D between groups Funding: BBSRC grant	D3 biscuit (n=67) Duration: 12 weeks Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: HPLC MS MS		decreased 25(OH)D2	effective in increasing total 25(OH)D. Decrease in 25(OH)D3 shown in aggregated vitamin D2 group (D2 biscuit and D2 juice combined). Study also showed a decrease in 25(OH)D2 in the D3 juice group
Biancuzzo and others (2010) (USA) Participants (n=86): Men (n=27) and women (n=59) (mean age 40.1±15.6 years) Objective: to compare bioavailability of vitamin D2 and D3 from orange juice with that from vitamin D2 and D3 supplements Study design: Double blind, controlled Commenced in February	Dose: 25 (1000) Groups: Placebo (n=15) D2 juice (n=17) D2 capsule (n=16) D3 juice (n=18) D3 capsule (n=20) Duration: 11 weeks Measurements: 25(OH)D Method of analysis: LCMS MS	No difference between D2 and D3	Data not provided	Vitamins D2 and D3 are equally bioavailable in orange juice and capsules Consumption of 25 µg (1000 IU) vitamin D2 or vitamin D3 in orange juice was equally as effective as 25 µg (1000 IU) vitamin D2 or D3 in capsule form in raising and maintaining total 25(OH)D concentrations

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
Study power: not reported Funding: NIH and Beverage Institute for Health and Wellness (Division of Coca-Cola, N America)				
Biancuzzo and others (2013) (USA) Participants (n=34): samples used from previous study – see above) Objective: To determine 1,25-dihydroxyvitamin D3 and 1,25-dihydroxyvitamin D2 levels in adults consuming 1000 IU vitamin D2 or D3 daily for 11 weeks Study design: Double blind, controlled Commenced in February Study power: not reported Funding: UV Foundation, Mushroom Council, NIH Clinical Translational Science Institute	Dose: 25 (1000) Groups: Placebo (n=8) D2 (n=17) D3 (n=9) Duration: 11 weeks Measurements: 25(OH)D2, 25(OH)D3 Method of analysis: LCMS MS	NA	No effect	Confirmed that there was no significant difference in the increase in serum 25(OH)D2 for the group who received vitamin D2 compared with the increase in 25(OH)D3 in the group who received vitamin D3. This was reflected by observation that the total serum 25(OH)D concentrations were no different for the groups ingesting vitamin D2 or D3
Glendenning and others (2009) (Australia)	Dose: 25 (1000) Groups:	D3 more effective than D2	D2 decreased 25(OH)D3	In elderly, hip fracture patients, supplementation with

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
Participants (n=70): Hip-fracture inpatients in hospital (mean age, 83±8 years) Objective: to determine if vitamin D2 and D3 are equipotent therapies Study design: Double-blind, uncontrolled Study power: n=37 per group required for 80% power (α=0.05) to detect 50% difference between-groups on 25(OH)D Funding: Royal Perth Hospital Medical Research Foundation	D2 (n=34) D3 (n=36) Duration: 3 months Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: HPLC			vitamin D3 was more effective than vitamin D2 in increasing total 25(OH)D concentration. However, all treatment-compliant patients achieved serum 25(OH)D concentrations above 50 nmol per litre irrespective of whether they received vitamin D2 or D3
Holick and others (2008) (USA) Participants (n=68): Men (n=21) and women (n=47) (age 18 to 84 years) Objective: to determine whether vitamin D2 less effective than vitamin D3 in maintaining serum 25(OH)D or increased catabolism of 25(OH)D3 Study design: Double blind, controlled	Dose: 25 (1000) Groups: Placebo (n=14) D2 (n=16) D3 (n=20) D2+D3 (n=18) (12.5 µg or 500 IU of each) Duration: 11 weeks Measurements: 25(OH)D,	No difference between D2, D3, or D2+D3 group	No effect	A 25 µg (1000 IU) dose of vitamin D2 daily was as effective as 25 µg (1000 IU) vitamin D3 in maintaining serum 25(OH)D concentrations and did not negatively influence serum 25(OH)D3 concentrations

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
Conducted in winter Study power: not reported Funding: NIH; Beverage Institute for Health and Wellness (Coca-Cola Co, Atlanta)	25(OH)D2, 25(OH)D3 Method of analysis: LCMS			
Itkonen and others (2016) (Finland) Participants (n=41): Women (age, 20 to 40 years) Objective: To investigate bioavailability of vitamin D2 fortified bread (from UV-irradiated baking yeast) versus D2 or D3 supplementation in raising serum 25(OH)D2, 25(OH)D3 and total 25(OH)D concentrations Study design: Single blind; controlled Conducted in winter (February to April) Study power: n=8 per group required for 90% power (α=0·05) to detect 25 (SD15) nmol per litre change between groups Funding: European Commission (7th Framework Programme)	Dose: 25 (1,000) Placebo (n=7) D2 supplement (n=9) D3 supplement (n=8) D2 bread (n=9) Duration: 8 weeks Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: LCMS MS	No difference between D2 and D3 supplement. No effect of D2 bread on 25(OH)D	D2 decreased 25(OH)D3. D3 had no effect on 25(OH)D2	Vitamin D2 is less potent in increasing total 25(OH)D concentration compared to vitamin D3 Vitamin D2 supplementation decreases 25(OH)D3 concentrations, indicating possible replacement of 25(OH)D3 by 25(OH)D2 in biological actions of vitamin D

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
Logan and others (2013) (New Zealand) Participants (n=62): Men (n=13) and women (n=49) (mean age, 29y, range, 18 to 50 years) Objective: To evaluate effect of a daily physiological dose of vitamin D2 or D3 on 25(OH)D concentration in healthy adults at the end of summer Study design: Double blind, controlled Conducted end of summer Study power: not reported Funding: Otago University Medical Research Fund	Dose: 25 (1000) Groups: Placebo (n=25) D2 (n=13) D3 (n=24) Duration: 25 weeks Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: LCMS	D3 more effective than D2	D2 decreased 25(OH)D3	Daily supplementation of 25µg (1,000 IU) vitamin D3 over 25 weeks was more effective than the same dose of vitamin D2 in maintaining serum 25(OH)D concentrations

Table A7.2: studies that administered vitamins D2 and D3 at daily doses less than 25µg (1000 IU) per day

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
Binkley and others (2011) (USA) Participants (n=32): Men (n=12) and women (n=20) (mean age 77 years; range 65 to 88 years) Objective: to evaluate effect of daily and once monthly dosing of D2 or D3 on 25(OH)D concentration Study design: Double blind, uncontrolled Conducted over 1 year Study power: Not reported Funding: GlaxoSmithKline	Dose: 40 (1600) Groups: D2 (n=16) D3 (n=16) Duration: 1 year Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: HPLC	D3 more effective than D2 No adverse effects reported	D2 decreased 25(OH)D3 concentration (data shown graphically). D3 decreased 25(OH)D2 concentration (data not reported)	Vitamin D3 produced a greater increment in serum 25(OH)D than vitamin D2 Supplementation with vitamin D2 led to a prompt and substantial ($p<0.0001$) decrease in circulating 25(OH)D3. Dosing with vitamin D3 appeared to reduce circulating 25(OH)D2 Vitamin D3 is slightly, but significantly, more effective than D2 at increasing 25(OH)D
Lehmann and others (2013) (Germany) Participants (n=107): Men (n=39) and women (n=68) (age, 19 to 67 years) Objective: to test effects of supplementation with vitamin D2 or	Dose: 50 (2000) Groups: Placebo (n=19) D2 (n=46) D3 (n=42) Duration: 8 weeks	D3 more effective than D2 No adverse effects reported	D2 decreased 25(OH)D3 concentration	Vitamin D3 increases the total 25(OH)D concentration more effectively than vitamin D2 Vitamin D2 supplementation causes a decrease in 25(OH)D3

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
D3 (50 µg/day) or a placebo on 25(OH)D2, 25(OH)D3 and total 25(OH)D Study design: Double blind, controlled Conducted in winter Study power: n=50 per group required for 80% power (α=0.05) to show 10 nmol per litre difference in total 25(OH)D between groups Funding: German Ministry of Education and Research	Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: LCMS			
Hartwell and others (1987) Denmark Participants (n=18): Women (age, 22 to 49 years) Objective: to examine and compare response of treatment with vitamin D2 and vitamin D3 on serum concentration of 1,25(OH)2D2 and 1,25(OH)2D3 in normal subjects Study design: Details of blinding not provided. Uncontrolled	Dose: 100 (4000) Groups: D2 (n=9) D3 (n=9) Duration: 8 weeks Measurements: 25(OH)D, 25(OH)D2, 25(OH)D3 Method of analysis: HPLC UV absorption	D3 more effective than D2. Did not report on adverse effects	D2 decreased 25(OH)D3 concentration	In vitamin D3 group the serum 25(OH)D3 and 25(OH)D increased significantly but was unchanged in the vitamin D2 treated group owing to an increased serum 25(OH)D2 and a correspondent decrease in serum 25(OH)D3. The data indicate that the 2 forms of vitamin D are metabolised differently

Study details	D2, D3 dose µg (IU), duration, metabolites measured and method	Effect of D2 versus D3 on total 25(OH)D concentration	Effect of D2 on 25(OH)D3 concentration	Authors' conclusion
Season study conducted - not reported Study power: Not reported Funding: Not reported				
Trang and others (1998) (Canada) Participants (n=72): Men (n=24) and women (n=48) (mean age, 38±9 years) Objective: to compare the ability of equal molar quantities of vitamin D2 or D3 to increase serum 25(OH)D Study design: 34 participants randomly assigned to D2 or D3 in double-blind manner; the rest assigned to D3, uncontrolled Conducted between February and early May Study power: Not reported Funding: Dairy Farmers of Canada	Dose: 100 (4000) Groups: D2 (n=17) D3 (n=55) No intervention (n=17) Duration: 14 days Measurements: 25(OH)D Method of analysis: RIA	D3 more effective than D2 Did not report on adverse effects	Not considered	Vitamin D3 more effective than vitamin D2 at raising serum 25(OH)D concentrations

Annex 8: daily doses of vitamin D3 or D2 and 25(OH)D concentrations

The table shows comparisons of daily doses of vitamin D3 or D2 with changes from baseline in 25(OH)D, 25(OH)D2 and 25(OH)D3 concentrations. Studies (from the Balachandar systematic review) are listed by ascending order of vitamin D dose.

First author (year), sample size	D3 or D2 dose µg (IU) per day and duration	Total 25(OH)D (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D2 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D3 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)
Fisk (2012) n=32	5 (200)	D3: 11.9 [11.06] D2: 4.9 [8.97]	7.00 [−2.87, 16.87]	D3: —0.1 [0.18 D2: 9.2 [4.43]	−9.30 [−12.37, −6.23]	D3: 12 [11.06] D2: —3.8 [7.83]	15.80 [6.41, 25.19]
Fisk (2012) n=32	10 (400) 4 weeks	D3: 19.7 [12.38] D2: 13.6 [11.3]	6.10 [−5.5, 17.72]	D3: 0 [0.12] D2: 17.6 [9.99]	−17.60 [−24.52, −10.68]	D3: 19.8 [12.44] D2: −2.9 [7.6]	22.70 [12.60, 32.80]
Nimitphong (2013) n=41	10 (400) 3 months	D3: 16.13 [8.8] D2: 7.84 [4.84]	8.29 [3.91, 12.67]	D3: −0.9 [2.05] D2: 21.89 [4.33]	−21.98 [−24.04, −19.92	D3: 16.22 [8.81] D2: −14.2 [3.93]	30.42 [26.21, 34.63]
Tripkovic (2017) n=270	15 (600) biscuit	D3: 30.03 [36.74] D2: 15.96	14.07 [1.33, 26.81]	D3: 0.01 [1.73] D2: 29.84 [32.39]	−29.83 [—37.66, —22.00]	D3: 34.19 [41.18] D2: −12.57	46.76 [35.21, 58.31]

First author (year), sample size	D3 or D2 dose µg (IU) per day and duration	Total 25(OH)D (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D2 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D3 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)
		[38.19]				[24.9]	
Tripkovic (2017) n=270	15 (600) juice 12 weeks	D3: 31.22 [35.08] D2: 13.8 [33.9]	17.42 [5.87, 28.97]	D3: -0.21 [1.54] D2: 29.13 [32.39]	−29.34 [–37.10, –21.58]	D3: 32.4 [42.26] D2: −16.31 [23.53]	48.71 [37.32, 60.10]
Biancuzzo (2010) n=71	25 (1,000) juice	D3: 31.95 [25.21] D2: 26.46 [17.97]	5.49 [−8.95, 19.93]	Not reported	Not reported	Not reported	Not reported
Biancuzzo (2010) n=71	25 (1,000) capsules 11 weeks	D3: 23.21 [17.72] D2: 26.96 [14.73]	−3.75 [−14.35, 6.85]	Not reported	Not reported	Not reported	Not reported
Biancuzzo (2013) n=26 (samples from previous study–see above)	25 (1,000) 11 weeks	Not reported (previously reported - see above)	Not applicable	D3: −2.18 [6.53] D2: 15.73 [13.31]	−17.91 [−25.54, −10.28]	D3: 30.75 (18.75) D2: 3.25 (28)	27.50 [9.41, 45.59]

First author (year), sample size	D3 or D2 dose µg (IU) per day and duration	Total 25(OH)D (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D2 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D3 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)
Glendenning (2009) n=95	25 (1,000) 3 months	D3: 39.76 [48.31] D2: 21.96 [25.11]	17.80 [2.27, 33.33]	D3: 4.58 [22.3] D2: 17.93 [31.72]	−13.35 [−24.36, −2.34]	D3: 29.07 [28.39] D2: −4.54 [18.1]	33.61 [24.01, 43.21]
Holick (2008) (note 1) n=36	25 (1,000) 11 weeks	D3: 24.75 [8) D2: 23.25 [17.74]	−1.5 [-10.2, 7.2]	D3: −0.33 [1.15] D2: 25.55 [13.05]	−25.88 [−32.3, −19.45]	D3: 23.08 [25.6] D2: −2.98 [34.8]	26.05 [5.63, 46.48]
Itkonen (2016) (note 2) n=21	25 (1,000) 8 weeks	D3: 17.0 D2: 9.6	7.4	D3: 0.01 [0.01] D2: 31.3 [0.1]	−31.20 [−31.29, –31.11]	D3: 18.5 [0.1] D2: –21.7 [0.1]	40.20 [40.11, 40.29]
Logan (2013) n=55	25 (1,000) 25 weeks	D3: 1.12 [22.97] D2: −17 [20.27]	18.12 [7.43, 28.81]	Not reported	Not reported	Not reported	Not reported

First author (year), sample size	D3 or D2 dose µg (IU) per day and duration	Total 25(OH)D (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D2 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)	25(OH)D3 (nmol per litre) [SD]	MD [95% CI] (nmol per litre)
Binkley (2011) n=32	40 (1600) I year	D3: 22.88 [8.52] D2: 15.08 [7.35]	7.80 [2.29, 13.31]	Not reported	Not reported	Not reported	Not reported
Lehmann (2013) n=88	50 (2000) 8 weeks	D3: 45.5 [21.7] D2: 30.2 [20.1]	15.30 [6.54, 24.06]	D3: 0.1 [0.1] D2: 43.7 [18.5]	43.60 [−48.95, −38.25]	D3: 46.5 [21.3] D2: −19.8 [9.6]	66.30 [59.29, 73.31]
Hartwell (1987) n=18	100 (4000) 8 weeks	D3: 32.57 [8.41] D2: 13.08 [10.94]	19.49 [10.47, 28.51]	D3: 0.1 [0.1] D2: 53.31 [8.67]	−53.21 [−58.87, -47.55]	D3: 33.27 [8.09] D2: −48.29 [6.17]	81.56 [74.91, 88.21]
Trang (1998) n=72	100 (4000) 14 days	D3: 23.3 [15.7] D2: 13.7 [11.4]	9.60 [2.77, 16.43]	Not reported	Not reported	Not reported	Not reported

Abbreviations. 25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; IU, international units; µg, micrograms; MD, mean difference; SD, standard deviation.

Note 1. Units of measurement in Holick and others (2008) paper were ng per ml and these were reported in the forest plot in Balachandar and others (2021); they have been converted here to nmol per litre. Unclear where the measurements for impact of D3 and D2 on 25(OH)D2 and 25(OH)D3 were from because these were presented graphically in the original paper.

Note 2. Mean changes in 25(OH)D concentration for D3 and D2 groups were incorrectly reported as +9.3 and +4.8 nmol per litre respectively by Balachandar and others (2021). The MD between D3 and D2 was reported incorrectly as 4.5 nmol per litre.

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