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Common variants in CYP2R1 and GC genes are both determinants of serum 25-hydroxyvitamin D concentrations after UVB irradiation and after consumption of vitamin D3-fortified bread and milk during winter in Denmark

Background: Little is known about how the genetic variation in vitamin D modulating genes influences ultraviolet (UV)B–induced 25-hydroxyvitamin D [25(OH)D] concentrations. In the Food with vitamin D (VitmaD) study, we showed that common genetic variants rs10741657 and rs10766197 in 25-hydroxylase (CYP2R1) and rs842999 and rs4588 in vitamin D binding protein (GC) predict 25(OH)D concentrations at late summer and after 6-mo consumption of cholecalciferol (vitamin D3)–fortified bread and milk.

Objectives: In the current study, called the Vitamin D in genes (VitDgen) study, we analyzed associations between the increase in 25(OH)D concentrations after a given dose of artificial UVB irradiation and 25 single nucleotide polymorphisms located in or near genes involved in vitamin D synthesis, transport, activation, or degradation as previously described for the VitmaD study. Second, we aimed to determine whether the genetic variations in CYP2R1 and GC have similar effects on 25(OH)D concentrations after artificial UVB irradiation and supplementation by vitamin D3–fortified bread and milk.

Design: The VitDgen study includes 92 healthy Danes who received 4 whole-body UVB treatments with a total dose of 6 or 7.5 standard erythema doses during a 10-d period in winter. The VitmaD study included 201 healthy Danish families who were given vitamin D3–fortified bread and milk or placebo for 6 mo during the winter.

Results: After UVB treatments, rs10741657 in CYP2R1 and rs4588 in GC predicted UVB-induced 25(OH)D concentrations as previously shown in the VitmaD study. Compared with noncarriers, carriers of 4 risk alleles of rs10741657 and rs4588 had lowest concentrations and smallest increases in 25(OH)D concentrations after 4 UVB treatments and largest decreases in 25(OH)D concentrations after 6-mo consumption of vitamin D3–fortified bread and milk.

Conclusion: Common genetic variants in the CYP2R1 and GC genes modify 25(OH)D concentrations in the same manner after artificial UVB-induced vitamin D and consumption of vitamin D3–fortified bread and milk. The VitDgen study was registered at clinicaltrials.gov as NCT01741233. The VitmaD study was registered at clinicaltrials.gov as NCT01184716.
Common Variants in CYP2R1 and GC Genes Predict Vitamin D Concentrations in Healthy Danish Children and Adults

Environmental factors such as diet, intake of vitamin D supplements and exposure to sunlight are known to influence serum vitamin D concentrations. Genetic epidemiology of vitamin D is in its infancy and a better understanding on how genetic variation influences vitamin D concentration is needed. We aimed to analyse previously reported vitamin D-related polymorphisms in relation to serum 25(OH)D concentrations in 201 healthy Danish families with dependent children in late summer in Denmark. Serum 25(OH)D concentrations and a total of 25 SNPs in GC, VDR, CYP2R1, CYP24A1, CYP27B1, C10or88 and DHCR7/NADSYN1 genes were analysed in 758 participants. Genotype distributions were in Hardy-Weinberg equilibrium for the adult population for all the studied polymorphisms. Four SNPs in CYP2R1 (rs1562902, rs7116978, rs10741657 and rs10766197) and six SNPs in GC (rs4588, rs842999, rs2282679, rs12512631, rs16846876 and rs17467825) were statistically significantly associated with serum 25(OH)D concentrations in children, adults and all combined. Several of the SNPs were in strong linkage disequilibrium, and the associations were driven by CYP2R1-rs10741657 and rs10766197, and by GC-rs4588 and rs842999. Genetic risk score analysis showed that carriers with no risk alleles of CYP2R1-rs10741657 and rs10766197, and by GC-rs4588 and rs842999 had significantly higher serum 25(OH)D concentrations compared to carriers of all risk alleles. To conclude, our results provide supporting evidence that common polymorphisms in GC and CYP2R1 are associated with serum 25(OH)D concentrations in the Caucasian population and that certain haplotypes may predispose to lower 25(OH)D concentrations in late summer in Denmark.
Real-life use of vitamin D₃-fortified bread and milk during a winter season: the effects of CYP2R1 and GC genes on 25-
hydroxyvitamin D concentrations in Danish families, the VitmaD study.

Common genetic variants rs10741657 and rs10766197 in CYP2R1 and rs4588 and rs842999 in GC and a combined
genetic risk score (GRS) of these four variants influence late summer 25-hydroxyvitamin D (25(OH)D) concentrations. The
objectives were to identify those who are most at risk of developing low vitamin D status during winter and to assess
whether vitamin D₃-fortified bread and milk will increase 25(OH)D concentrations in those with genetically determined low
25(OH)D concentrations at late summer. We used data from the VitmaD study. Participants were allocated to either
vitamin D₃-fortified bread and milk or non-fortified bread and milk during winter. In the fortification group, CYP2R1
(rs10741657) and GC (rs4588 and rs842999) were statistically significantly associated with winter 25(OH)D
concentrations and carriage of 0-8 risk alleles (p <0.0001). No association was found for the control group (p = 0.1428).
There was a significant positive linear relationship between different quintiles of total vitamin D intake and the increase in
25(OH)D concentrations among carriers of 0-2 (p = 0.0012), 3 (p = 0.0001), 4 (p = 0.0118) or 5 (p = 0.0029) risk alleles,
but not among carriers of 6-8 risk alleles (p = 0.1051). Carriers of a high GRS were more prone to be vitamin D deficient
compared to carriers of a low GRS. Furthermore, rs4588-AA carriers have a low but very stable 25(OH)D concentration,
and interestingly, also low PTH level.
Vitamin D status and effects of food fortification in families

Background and aims: The importance of vitamin D in bone health is recognised and low concentrations have been associated with increased risk of disease. Cutaneous synthesis is considered the major source of vitamin D, but during winter where sufficient sun exposure is restricted at Northern latitudes, intake from food and dietary supplements become essential. Vitamin D intakes are lower than dietary recommendations in most populations and low vitamin D status is common. The PhD thesis is based on the VitmaD study in which a realistic and model derived fortification strategy was investigated in a real-life setting. The aim was to investigate the effect of increasing vitamin D intake by fortification of milk and bread to the amount recommended in the Nordic Nutrition recommendations (NNR) on serum 25(OH)D concentration in families during winter in Denmark (paper 1). Secondly, the aim was to assess vitamin D status and its determinants at baseline of the study (paper 2). Further, to model the relationship between total vitamin D intake and serum 25(OH)D taking into account potential effect modifiers and estimate required vitamin D intake during winter (paper 3).

Methods: The VitmaD study was a randomized controlled trial in 782 children and adults (4-60 years) recruited as 201 families. Families
were randomly assigned to vitamin D fortified or nonfortified milk and bread for 6 months starting from September. The milk and bread replaced the subjects’ usual consumptions of products. Information on dietary intake, supplement use, health and lifestyle was obtained by self-administered web-based questionnaires. Serum 25(OH)D was analysed by liquid chromatography-tandem mass spectrometry (LC/MS-MS). Mixed models with family as a random factor were applied in all the statistical analyses. Results: At baseline of the study (late summer) the geometric mean (IQR) serum 25(OH)D concentration was 72.1 (61.5-86.7) nmol/L with no overall differences between age (P=0.190), gender (P=0.332) or age and gender groups (P=0.223) (paper 2). The prevalence of serum 25(OH)D <50 nmol/L was 9 %. In the multiple analysis of all subjects, vitamin D status was negatively associated with BMI (P<0.001) and positively associated with dietary vitamin D (P=0.008), multivitamin use (P=0.019), solarium use (P=0.006), outdoor stay in light clothes (P=0.001), sun preference (P=0.002) and sun vacation (P<0.001). The intra-family correlation was stronger in children (0.42) compared with adults (0.24). Thus children within a family seemed to be more alike than adults within a family with respect to vitamin D status. The planned fortification strategy was to increase the vitamin D intake to 7.5 µg/day. This succeeded in 66 % of the subjects in the fortification group with a median vitamin D intake (habitual diet plus fortified milk and bread) of 9.4 µg/day compared with 2.2 µg/day in the control group (paper 1). During winter the serum 25(OH)D concentration decreased from 73.1 to 67.6 nmol/L (-Δ5.5 nmol/L) in the fortification group (P<0.001) and from 71.1 to 41.7 nmol/L (-Δ29.4 nmol/L) in the control group (P<0.001). The final serum 25(OH)D concentration was significantly higher in the fortification group compared with in the control group (P<0.001, interpreted estimate 1.59) and the treatment effect was not affected by BMI, multivitamin use and sun vacation. The prevalence of serum 25(OH)D <50 nmol/L remained low in the fortification group (16 %) whereas it increased to 65 % in the control group. The relationship between total vitamin D intake from natural foods, fortified milk and bread and dietary supplements and serum 25(OH)D concentration in winter was best fitted by a non-linear curve (paper 3). The effect of total vitamin D intake on serum 25(OH)D concentration was 4 % higher in men compared with women (P<0.014) and 10 % higher in the group with lowest initial 25(OH)D concentration (<61.5 nmol/L) compared with the group with highest initial 25(OH)D concentration (>86.9 nmol/L) (P<0.001). It was not modified by age (P=0.132) or BMI (P=0.884). Estimated required vitamin D intake was 5, 11, 23 and 39 µg/day for 50, 75, 90 and 95 % of the population to maintain vitamin D status >50 nmol/L during winter. These figures were higher for the group with lowest initial 25(OH)D concentration (11, 18, 34 and >34 µg/day) and lower for the group with highest initial 25(OH)D concentration (<1, 3, 8 and 17 µg/day). Conclusions: In the population of Danish families, serum 25(OH)D concentration was above 50 nmol/L in late summer and it was associated with both dietary and sun related factors. Children within a family seemed to be more alike than adults within a family with respect to vitamin D status. Vitamin D fortification of milk and bread reduced the decrease in serum 25(OH)D concentration during winter and ensured concentrations above 50 nmol/L. The relationship between total vitamin D intake and vitamin D status was non-linear. Estimated total vitamin D intake to maintain serum 25(OH)D above 50 nmol/L was largely dependent on the initial vitamin D status.

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Vitamin D status and its determinants in children and adults among families in late summer in Denmark.

The impact of the familial relationship on vitamin D status has not been investigated previously. The objective of the present cross-sectional study was to assess serum 25-hydroxyvitamin D (25(OH)D) concentration and its determinants in children and adults among families in late summer in Denmark (56°N). Data obtained from 755 apparently healthy children (4-17 years) and adults (18-60 years) recruited as families (n 200) in the VitmaD study were analysed. Blood samples were collected in September-October, and serum 25(OH)D concentration was measured by liquid chromatography-tandem MS. Information on potential determinants was obtained using questionnaires. Serum 25(OH)D was analysed by liquid chromatography-tandem mass spectrometry (LC/MS-MS). Mixed models with family as a random factor were applied in all the statistical analyses. Results: At baseline of the study (late summer) the geometric mean (IQR) serum 25(OH)D concentration was 72.1 (61.5-86.7) nmol/L with no overall differences between age (P=0.190), gender (P=0.332) or age and gender groups (P=0.223) (paper 2). The prevalence of serum 25(OH)D <50 nmol/L was 9 %. In the multiple analysis of all subjects, vitamin D status was negatively associated with BMI (P<0.001) and positively associated with dietary vitamin D (P=0.008), multivitamin use (P=0.019), solarium use (P=0.006), outdoor stay in light clothes (P=0.001), sun preference (P=0.002) and sun vacation (P<0.001). The intra-family correlation was stronger in children (0.42) compared with adults (0.24). Thus children within a family seemed to be more alike than adults within a family with respect to vitamin D status. The planned fortification strategy was to increase the vitamin D intake to 7.5 µg/day. This succeeded in 66 % of the subjects in the fortification group with a median vitamin D intake (habitual diet plus fortified milk and bread) of 9.4 µg/day compared with 2.2 µg/day in the control group (paper 1). During winter the serum 25(OH)D concentration decreased from 73.1 to 67.6 nmol/L (-Δ5.5 nmol/L) in the fortification group (P<0.001) and from 71.1 to 41.7 nmol/L (-Δ29.4 nmol/L) in the control group (P<0.001). The final serum 25(OH)D concentration was significantly higher in the fortification group compared with in the control group (P<0.001, interpreted estimate 1.59) and the treatment effect was not affected by BMI, multivitamin use and sun vacation. The prevalence of serum 25(OH)D <50 nmol/L remained low in the fortification group (16 %) whereas it increased to 65 % in the control group. The relationship between total vitamin D intake from natural foods, fortified milk and bread and dietary supplements and serum 25(OH)D concentration in winter was best fitted by a non-linear curve (paper 3). The effect of total vitamin D intake on serum 25(OH)D concentration was 4 % higher in men compared with women (P<0.014) and 10 % higher in the group with lowest initial 25(OH)D concentration (<61.5 nmol/L) compared with the group with highest initial 25(OH)D concentration (>86.9 nmol/L) (P<0.001). It was not modified by age (P=0.132) or BMI (P=0.884). Estimated required vitamin D intake was 5, 11, 23 and 39 µg/day for 50, 75, 90 and 95 % of the population to maintain vitamin D status >50 nmol/L during winter. These figures were higher for the group with lowest initial 25(OH)D concentration (11, 18, 34 and >34 µg/day) and lower for the group with highest initial 25(OH)D concentration (<1, 3, 8 and 17 µg/day). Conclusions: In the population of Danish families, serum 25(OH)D concentration was above 50 nmol/L in late summer and it was associated with both dietary and sun related factors. Children within a family seemed to be more alike than adults within a family with respect to vitamin D status. Vitamin D fortification of milk and bread reduced the decrease in serum 25(OH)D concentration during winter and ensured concentrations above 50 nmol/L. The relationship between total vitamin D intake and vitamin D status was non-linear. Estimated total vitamin D intake to maintain serum 25(OH)D above 50 nmol/L was largely dependent on the initial vitamin D status.
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Randomized controlled trial of the effects of vitamin D–fortified milk and bread on serum 25-hydroxyvitamin D concentrations in families in Denmark during winter: the VitmaD study1-3

Background: Vitamin D intakes are lower than dietary recommendations in most populations, and thus, a low vitamin D status is widespread, especially during winter.

Objective: We investigated the effects of increasing vitamin D intake to the recommended amount by fortification of milk and bread on serum 25-hydroxyvitamin D [25(OH)D] concentrations in families during winter in Denmark.

Design: The study was a randomized controlled trial in 782 children and adults (4–60 y old) recruited as 201 families. Families were randomly assigned to vitamin D–fortified or nonfortified milk and bread for 6 mo starting in September. The milk and bread replaced the participants’ usual consumptions of products.

Results: Median (IQR) vitamin D intakes (habitual diet plus fortified products) were 9.4 μg/d (6.5, 12.3 μg/d) and 2.2 μg/d (1.5, 3.0 μg/d) in fortification and control groups, respectively. Geometric mean (IQR) serum 25(OH)D concentrations decreased from 73.1 nmol/L (61.9, 88.5 nmol/L) to 67.6 nmol/L (56.2, 79.4 nmol/L) in the fortification group and from 71.1 nmol/L (61.2, 85.9 nmol/L) to 41.7 nmol/L (29.5, 58.9 nmol/L) in the control group (both P < 0.001). The final 25(OH)D concentration was significantly higher in the fortification group than in the control group (P < 0.001). By the end of the study, <1% of subjects in the fortification group and 25% of subjects in the control group had 25(OH)D concentrations <30 nmol/L and 16% and 65% of subjects, respectively, had 25(OH)D concentrations <50 nmol/L.

Conclusion: Vitamin D fortification of milk and bread reduces the decrease in serum 25(OH)D concentrations during winter and ensures 25(OH)D concentrations >50 nmol/L in children and adults in Denmark. This trial was registered at clinicaltrials.gov as NCT011184716.

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Vitamin D status among families in Denmark: Baseline data from the vitmad study

Background and objectives:
The beneficial effect of vitamin D in bone health is acknowledged and the vitamin has also been associated with several chronic diseases. It is therefore relevant to determine the prevalence of vitamin D insufficiency in different groups, and vitamin D statuses within families have not been studied previously. The objective of the present study was to evaluate serum 25-hydroxyvitamin D (25(OH)D) concentrations among families in Denmark (56 °N) after seasonal UVB peak and to ascertain determining factors.

Methods:
Cross-sectional study with 755 children and adults (4-60 y) recruited as families in the VitmaD study. Blood samples were collected in September-October 2010, and vitamin D status was measured as serum 25(OH)D concentration by LC-MS/MS. Vitamin D intake and life style factors were assessed in self-administered questionnaires. Determinants of vitamin D status were identified in a linear mixed model with family as a random variable.

Results:
Mean (±SD) serum 25(OH)D concentration was 75 ± 20 nmol/l (range 9-162 nmol/l) and only 10 % had 25(OH) D <50 nmol/l. Determinants of serum 25(OH)D were age (p=0.036), BMI class (p=0.001), multi vitamin use (p=0.033), sun behaviour (p=0.005), outdoor stay (p=0.033), sun vacation (p<0.001), and physical activity (p=0.040). Gender (p=0.692) and vitamin D intake (p=0.238) were not associated to serum 25(OH)D.

Conclusions:
The prevalence of vitamin D insufficiency among families in Denmark was low after seasonal UVB peak. Sun vacation was the strongest determinant for vitamin D status at this time of the year.

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OrgTrace – No difference found in bioactive compounds of organic and conventional crops

The objective of the present study was to compare the content of selected bioactive compounds in organically and conventionally grown crops, and to evaluate if the ability of the crops to synthesize selected secondary metabolites was systematically affected by growth systems across different growth years as well as soil types. The results showed that contents of neither polyacetylenes and carotenoids in carrots, flavonoids in onions, nor phenolic acids in carrots and potatoes were significantly influenced by growth system. Thus it could not be concluded that the organically grown crops had higher contents of bioactive compounds than the conventionally grown. This indicates that giving preference to organic products because they contain more bioactive components is doubtful. However, there are many other reasons for the consumer to choose organic food products, including: no pesticide residues in foods, animal welfare, and environmental protection.

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Projects:
Vitamin D fortification
Several studies have shown that vitamin D status in the general population gradually decreases over the Winter season. This project aims at studying the effectiveness of vitamin D fortification of commonly consumed foods in alleviating this reduction in vit D status in families.

Division of Nutrition
National Food Institute
Arla Foods
Lantmannen
Øresund Food Network
Danish Agency for Science Technology and Innovation, Ministry of Science Innovation and Higher Education

Activities:

10th Nordic Nutrition Conference
Period: 2012 → …
Katja Howarth Madsen (Participant)
National Food Institute
Division of Nutrition
Description
Oral presentation

Related event

10th Nordic Nutrition Conference
03/06/2012 → 05/06/2012
Reykjavik, Iceland
Activity: Attending an event › Participating in or organising a conference

2nd International Vitamin Conference
Period: 2012
Katja Howarth Madsen (Participant)
National Food Institute
Division of Nutrition

Description
Poster presentation (The Danish vitamin D fortification study - VitmaD)

Related event

2nd International Vitamin Conference: Vitamins in Foods and Supplement - Analytical Challenges in Human Nutrition
23/05/2012 → 25/05/2012
Copenhagen, Denmark
Activity: Attending an event › Participating in or organising a conference

Press clippings:

Ph.d. projekt angående D-vitaminberigelse
Katja Howarth Madsen
23/01/2014
National Food Institute

Media contribution (1)

Ph.d. projekt angående D-vitaminberigelse
23/01/2014
Videnskab.dk, Web
Bo Christensen
Katja Howarth Madsen
National Food Institute
Press / Media

D-vitaminberigelse
Katja Howarth Madsen
28/02/2013
National Food Institute, Division of Nutrition

Media contribution (1)

D-vitaminberigelse
28/02/2013
Berlingske Tidende, Print
Christian Nørgaard Larsen
Katja Howarth Madsen
National Food Institute, Division of Nutrition
Press / Media

D-vitaminberigelse
Katja Howarth Madsen
27/02/2013
National Food Institute, Division of Nutrition
D-vitaminberigelse
27/02/2013
DR, Web
Bjørn Schiøening
Katja Howarth Madsen
National Food Institute, Division of Nutrition
Press / Media

D-vitamin berigelse
Katja Howarth Madsen
01/02/2013
National Food Institute, Division of Nutrition
Press / Media

D-vitamin berigelse
Katja Howarth Madsen
31/01/2013
National Food Institute, Division of Nutrition
Press / Media