BMJ Open Diabetes Research & Care

Effect of vitamin D_3 supplementation on glycated hemoglobin (HbA1c), fructosamine, serum lipids, and body mass index: a randomized, double-blinded, placebo-controlled trial among healthy immigrants living in Norway

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To cite: Madar AA, Knutsen KV, Stene LC, *et al*. Effect of vitamin D_3 supplementation on glycated hemoglobin (HbA1c), fructosamine, serum lipids, and body mass index: a randomized, double-blinded, placebo-controlled trial among healthy immigrants living in Norway. *BMJ Open Diabetes Research and Care* 2014;**2**:e000026. doi:10.1136/bmjdrc-2014-000026

► Additional material is available. To view please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2014-000026).

Received 20 February 2014
Revised 26 May 2014
Accepted 4 August 2014

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ABSTRACT

Objective: Despite the suggested role of vitamin D in the prevention of diabetes and cardiovascular disease or its risk factors, the evidence is not consistent and there is a paucity of randomized controlled trials in this field. We aimed to investigate the effect of 16-week daily vitamin D_3 supplementation on glycated hemoglobin (HbA1c), fructosamine, body mass index (BMI), and serum lipids.

Design: Double-blind, randomized, placebo-controlled trial.

Setting: Immigrant community centers in Oslo, Norway.

Participants: 251 healthy adults aged 18–50 years with a non-Western immigrant background. All participants performed the baseline test and 215 (86%) returned to the follow-up test.

Intervention: 16 weeks of daily oral supplementation with either 10 μg vitamin D_3, 25 μg vitamin D_3, or placebo.

Main outcome measures: Difference in absolute change during the 16-week intervention between the intervention groups combined (10 or 25 μg of vitamin D_3/day) and placebo, in HbA1c, fructosamine, serum lipids (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglycerides), and BMI.

Results: A total of 215 (86%) participants completed the study. Serum 25-hydroxyvitamin D increased from 29 nmol/L at baseline to 49 nmol/L after intervention, with little change in the placebo group. However, there was no difference in change of HbA1c between those receiving vitamin D_3 compared with placebo (mean difference: 0.01% (95% CI −0.04 to 0.06, p=0.7)). Neither did the vitamin D_3 supplementation have any effect on the other end points: fructosamine, serum lipids, and BMI.

Conclusions: 16-week vitamin D_3 supplementation to healthy immigrants from South Asia, the Middle East, or Africa and now living in Norway with low vitamin D status did not improve HbA1c, fructosamine, lipid profiles, or BMI. An updated meta-analysis of similar published trials showed that our results were generally consistent with those of other studies.

Trial registration number: NCT01263288.

Key messages

- Immigrants are at risk both for viatmin D deficiency, overwieght and diabetes type-2.
- Effective and specific preventive means for vitamin D deficiency, overweight and risk of diabetes in immigrant population should be explored.
- More studies are needed to study the long-term health consequences of the low vitamin D status of immigrants.

INTRODUCTION

Vitamin D deficiency is widespread among immigrants from non-Western countries in Europe and elsewhere.[1–4] Classical outcomes of severe vitamin D deficiency are rickets in children and osteomalacia in adults. Low vitamin D status is also a risk factor for low bone density and osteoporotic fractures. Furthermore, during the past decades, the vitamin D receptor has been found in many tissues and new actions of vitamin D have been described.[5]

Immigrants, particularly those from South Asia and the Middle East, are at high risk of type 2 diabetes[6–8] and have a high prevalence of cardiovascular risk factors such as unfavorable serum lipids.[9 10] Several epidemiological studies have suggested increased risks of diabetes or impaired glucose metabolism and cardiovascular diseases among persons with low vitamin D status.[11–14]

Despite the suggested role of vitamin D in the prevention of diabetes and cardiovascular disease, the evidence is not consistent and there is a paucity of randomized controlled trials (RCTs) assessing the effect of vitamin D supplementation on these outcomes. In particular, few studies have been carried out on immigrants living in developed countries, who are at increased risk of both hypovitaminosis D and diabetes.[6] To study whether vitamin D supplementation has an effect on glucose metabolism, serum lipids and body mass index (BMI) in healthy adults with an immigrant background in Norway, we have carried out a randomized, double-blinded, controlled trial.

Results from the primary end point have previously been reported.[15] We present results from predefined additional end points. The aim was to test whether 16 weeks of daily vitamin D3 supplementation (10 or 25 µg/day vs placebo) would reduce glycated hemoglobin (HbA1c), fructosamine,and BMI and improve serum lipids.

RESEARCH DESIGN AND METHODS

Study design and participants

The study was conducted between January and June 2011 and further details of the study method have been described elsewhere.[15] Participants were healthy men and women, aged 18–50 years, who were born or had parents born in the Middle East, Africa, and South Asia. They were recruited through 11 different community centers in Oslo and surrounding areas (at latitude 60°N). They were excluded if they regularly used vitamin D-containing supplements, were receiving treatment for vitamin D deficiency, were pregnant or breastfeeding, used medication for hypoglycemia or hyperlipidemia, malabsorption, used medication interfering with the vitamin D metabolism (such as thiazides, antiepileptic drugs, prednisolone, or hormone replacement therapy), had kidney disease, cancer, tuberculosis, sarcoidosis, osteoporosis, or a recent fracture, or used strong painkillers prescribed by a physician such as Paralgin forte and Aporex. The same data collection team visited all the centers and performed the baseline and follow-up data collection. Interpreters were used when necessary, but the majority of the study participants were able to communicate in the Norwegian language.

RANDOMIZATION AND INTERVENTION

Those who fulfilled the eligibility criteria were randomly assigned to one of three equally sized intervention groups receiving one tablet per day containing 25 µg vitamin D_3, 10 µg vitamin D_3, or placebo. The tablets were similar in color, size, and packing. Each study participant was given a box of 120 tablets (a 16-week use corresponds to 112 tablets) at baseline with a self-administered compliance form. The tablets were manufactured by Bioplus Life Sciences Pvt Ltd, DMA (Bangalore, India), certified for Good Manufacturing Practice, and the ingredients met the requirements of British Pharmacopé. If the study participants had forgotten to take one tablet a day, they were asked to take two tablets the following day. The participants were followed up with a short text message twice a week to remind them to take the tablets. They were advised to maintain their usual dietary pattern during the 16-week trial period and contact the study staff by telephone if they had any inquiries.

MAIN OUTCOME VARIABLES

The study outcomes were difference in absolute change during the 16-week intervention between the intervention groups combined (10 or 25 µg of vitamin D_3/day) and placebo, in HbA1c, fructosamine, serum lipids (total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides), and BMI. HbA1c is correlated with the average glucose level over the preceding 2–3 months,[16] and it can be measured in non-fasting blood samples. Fructosamine reflects the average blood glucose level over the preceding 2–3 weeks, and the assay is not influenced by hemoglobinopathies or iron deficiency anemia that may influence HbA1c measurements.[17]

BLOOD SAMPLING AND LABORATORY ASSAYS

Non-fasting venous blood was drawn at baseline and at the follow-up after 16 weeks. Blood for serum was collected in serum-separator gel tubes and centrifuged after 30 min to 2 h, and blood for plasma was collected in EDTA tubes and centrifuged within 30 min at room temperature at the study site. Serum and plasma were separated and frozen in several aliquots at −20°C the same day and within 1–2 weeks frozen at −80°C until they were analyzed. After the completion of the study, all serum samples from baseline and follow-up were analyzed in one batch at the Fürst Medical Laboratory (http://www.furst.no), which is accredited by the International Organization for Standardization and is part of the vitamin D External Quality Assessment Scheme (DEQAS).

Serum 25-hydroxyvitamin D (s-25(OH)D) was measured using high-pressure liquid chromatography tandem mass spectrometry, with Waters Acquity UPLC and Waters triple quadrupole mass spectrometer instruments. Both 25(OH)D_2 and 25(OH)D_3 levels were measured and the sum of the two was used for analysis (termed 25(OH)D, even though 25(OH)D_2 was negligible). The within-batch coefficient of variation for s-25(OH)D_3 was 4.8% within high concentrations and 7.2% within low concentrations.

HbA1c was analyzed on a cation exchange column chromatograph using an automated high-pressure liquid chromatography instrument (HLC-723 G7, Tosoh Corporation, Tokyo, Japan). The reference upper normal for HbA1c is <6.1%. The total coefficient of variation was 2% at HbA1c levels around 6.1%.

Fructosamine was measured using a colorimetric enzymatic method (ADVIA 2400 Siemens). The interassay coefficient of variation (CV) was 2.3% and reference upper normal was 285 µmol/L.

Total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were measured using an enzymatic method (ADVIA 2400 Siemens). The interassay coefficients of variation were 1.3% (total cholesterol), 1.6% (LDL-cholesterol), 1.8% (HDL-cholesterol), and 3.8% (triglycerides).

Body weight was measured with a Bosogramm 3000 Scale (loading capacity 150 kg) to the nearest 100 g with participants in indoor clothing without shoes. Height was measured to the nearest centimeter with a rigid meter standard. The same devices were used both at baseline and at follow-up. The participants completed an interviewer-administered questionnaire at baseline and follow-up. Information about age, ethnicity, education, and duration of residence in Norway was collected at baseline.

RANDOM ALLOCATION

We chose a computer-generated block randomization to ensure a good balance of the number in each group during the trial and randomly varied the block size between 3 and 6.

BLINDING

Group allocation was unknown to participants, research staff, investigators, and data collectors. Data analyses were also blinded. The tablet boxes were numbered according to the randomization list by an external pharmacy (the Hospital Pharmacy at Oslo University Hospital). The group allocation list was stored at this pharmacy with a copy in a sealed envelope. Each participant was consecutively numbered and received a prepackaged tablet box with the corresponding number. The analyses of the primary outcome measures and the evaluation of the physical performance tests were performed before the randomization list was opened. At the end, the results of s-25(OH)D and plasma parathyroid hormone (PTH) analyses at follow-up were unmasked.

REGISTRATION

The study was authorized as a clinical trial by the Norwegian Medicine Agency. It has been registered at EudraCT (2010-021114-36). The clinical trial was conducted according to the principles of the Declaration of Helsinki and in accordance with national laws (ClinicalTrials.gov identifier NCT01263288).

STATISTICAL ANALYSES

The sample size was planned for an effect of the intervention on muscle strength and power. This was the main end point of the trial, and these results are presented elsewhere.[15] This suggested that we should include 210 participants, and under the assumption of expected dropout rates of 15–20% we aimed to recruit at least 250 participants. We also calculated that this sample size would provide 80% power to detect a difference of 0.5 (% points) in HbA1c between the intervention and control groups, a difference we considered clinically relevant.[18]

Statistical analysis of the data was performed using the IBM SPSS statistical software (V.19.0; SPSS Inc, Chicago, Illinois, USA). For each of the outcome variables, we calculated the difference in change from baseline to follow-up between the combined intervention groups (10 or 25 µg/day) and the placebo group. This was analyzed using linear regression analysis, where the effect on each outcome variable was adjusted for the respective baseline concentration. Similar analyses were also performed to compare 25 µg/day to placebo and 10 µg/day to placebo. p Values <0.05 were considered statistically significant.

Subgroup analyses by baseline values of end point measures, gender, and intervention dose were also performed.

All participants provided written consent. The participants were instructed that they would receive a notification by letter, if the analysis of their blood sample later showed deviant values with a recommendation to contact their regular general practitioners.

Role of the funding source

Nycomed, which supplied study tablets and other sponsors, had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

RESULTS

Participants

We screened 301 persons for inclusion in the study, of whom 251 met the inclusion criteria and agreed to participate. They were randomly assigned to one of the three intervention groups. After 16 weeks, 215 (86%) study participants returned to the follow-up visit, including one who declined to take the blood sample (figure 1).

Baseline characteristics

No substantial between-group differences in baseline values were noted (table 1). The mean baseline s-25(OH)D concentration for the whole study population was 28.9 (SD 17.6) nmol/L and 90% had s-25(OH)D <50 nmol/L while 53% had s-25(OH)D <25 nmol/L. Around 38% had HbA1c levels equal to or above 5.7%. The baseline correlation between HbA1c and serum fructosamine was 0.47 (p<0.001). There was no significant difference in baseline variables in the participants who did not complete the study (n=37) compared with those completed the study (n=214).

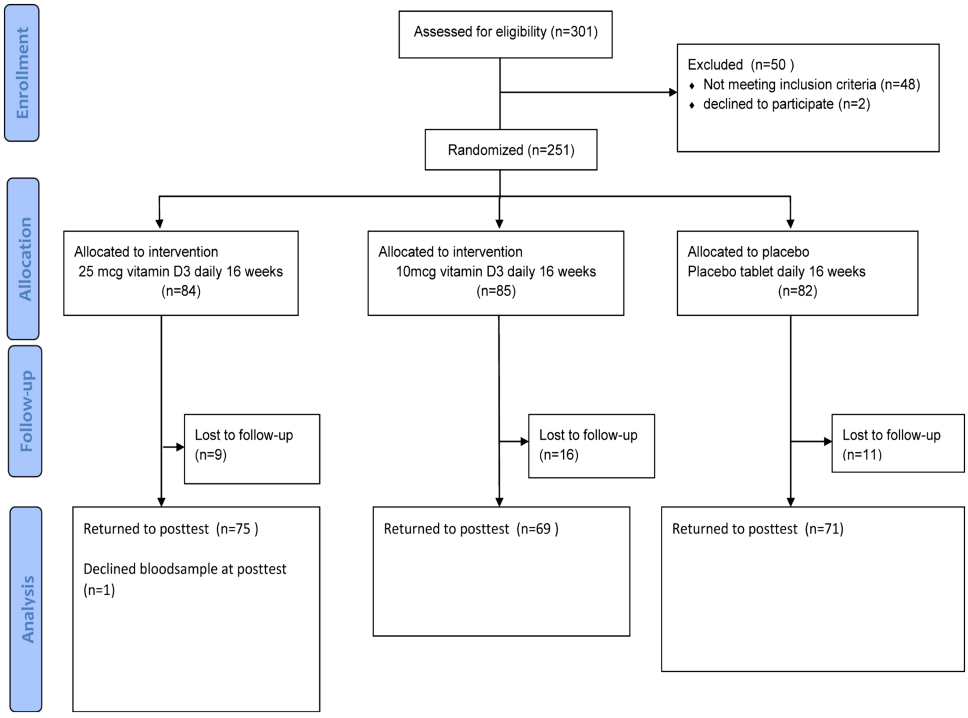

Figure 1 Flow chart of recruitment, randomization, and follow-up.

Table 1 Baseline characteristics for study participants by supplementation group*

Characteristics	Vitamin D (25 µg) N=84	Vitamin D (10 µg) N=85	Placebo N=82
Age (years)	36 (8.2)	37 (7.6)	39 (7.6)
Sex (n, %)			
Male	26 (31)	24 (28)	19 (23)
Female	58 (69)	61 (72)	63 (77)
Regional origin (n, %)			
South Asia	31 (37)	31 (36)	33 (40)
Middle East and North Africa	15 (18)	9 (11)	12 (15)
Sub-Saharan Africa	38 (45)	45 (53)	37 (45)
Years lived in Norway (mean, range)	13.3 (1–29)	13.2 (1–35)	13.6 (2–33)
Level of education, years (n, %)			
≤10	34 (40)	36 (42)	33 (40)
11–13	30 (36)	31 (37)	34 (42)
≥14	20 (24)	18 (21)	15 (18)
S-25(OH)D (nmol/L)	26.9 (16.5)	29.8 (20.6)	30.1 (18.9)
HbA1c (%)†	5.6 (0.65)	5.6 (0.51)	5.6 (0.43)
Fructosamine (µmol/L)	251 (50.5)	248 (35.9)	245 (30.1)
Total cholesterol (mmol/L)	4.8 (0.79)	4.9 (0.82)	4.9 (0.90)
HDL-cholesterol (mmol/L)	1.4 (0.31)	1.4 (0.31)	1.4 (0.32)
LDL-cholesterol (mmol/L)	3.3 (0.85)	3.3 (0.88)	3.4 (0.86)
Triglycerides (mmol/L)	1.6 (0.91)	1.5 (1.2)	1.6 (1.13)
BMI (kg/m^2)	26.9 (5.2)	27.5 (5.2)	27.8 (5.0)

*Data are mean (SD) unless specified otherwise.
†N=246. Thirty-seven participants did not come back to follow-up; 11 in placebo, 16 in the 10 µg group, and 10 in the 25 µg group. The baseline characteristics of these were not different from those who completed the study.
BMI, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; S-25(OH)D, serum 25-hydroxyvitamin D.

Table 2 Effect of vitamin D supplementation* on markers of glucose metabolism, serum lipids, and body mass index

	Baseline‡	After 16 weeks‡	Change from baseline to 16 weeks‡	Difference (95% CI) compared to placebo†	p Value
S-25(OHD) (nmol/L)					
Intervention (n=143)	28.7 (18.6)	48.8 (19.6)	20.1 (21.7)	21.3 (16.7 to 26.0)	<0.0001
Placebo (n=71)	29.2 (15.6)	27.5 (13.7)	−1.5 (11.3)		
HbA1c (%)§					
Intervention (n=139)	5.65 (0.6)	5.68 (0.7)	0.03 (0.17)	0.01 (−0.04 to 0.06)	0.7
Placebo (n=70)	5.52 (0.4)	5.52 (0.5)	0.01 (0.18)		
S-fructosamine (µmol/L)					
Intervention (n=143)	251 (45.9)	249.5 (58.4)	−1.1 (27.6)	1.7 (−5.3 to 8.7)	0.6
Placebo (n=71)	245 (30.8)	242.8 (29.9)	−3.2 (16.5)		
Total cholesterol (mmol/L)					
Intervention (n=143)	4.9 (0.8)	4.8 (0.8)	−0.1 (0.5)	−0.03 (−0.17 to 0.12)	0.7
Placebo (n=71)	4.9 (0.9)	4.8 (0.8)	−0.1 (0.5)		
LDL-cholesterol (mmol/L)					
Intervention (n=143)	3.3 (0.8)	3.2 (0.8)	−0.06 (0.4)	−0.01 (−0.1 to 0.1)	0.9
Placebo (n=71)	3.3 (0.8)	3.3 (0.8)	−0.05 (0.5)		
HDL-cholesterol (mmol/L)					
Intervention (n=143)	1.4 (0.3)	1.37 (0.3)	−0.01 (0.2)	−0.002 (−0.05 to 0.05)	0.9
Placebo (n=71)	1.4 (0.3)	1.39 (0.3)	−0.02 (0.2)		
Triglycerides (mmol/L)					
Intervention (n=143)	1.6 (1.1)	1.4 (0.8)	−0.18 (0.9)	0.03 (−0.2 to 0.20)	0.7
Placebo (n=71)	1.5 (1.1)	1.3 (0.7)	−0.16 (0.9)		
BMI (kg/m^2)					
Intervention (n=143)	26.9 (4.9)	26.9 (5.1)	−0.03 (0.6)	−0.05 (−0.2 to 0.1)	0.6
Placebo (n=71)	28.1 (5.2)	28.1 (5.2)	0.02 (0.7)		

*Combined 10 and 25 µg doses of vitamin D.
†Adjusted for baseline values.
‡Data are mean (SD) unless specified otherwise.
§N=209. Five participants had insufficient amount of blood for HbA1c measurements. The mean age of these was 32 years and the s-25(OH)D was 36.4 nmol/L (16.7), three persons were in the 10 µg group while the others were in the two other groups each.
BMI, body mass index; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; S-25(OH)D, serum 25-hydroxyvitamin D.

Effect of supplementation on vitamin D status

S-25(OH)D increased from a mean of approximately 29 nmol/L at baseline to 49 nmol/L after intervention, with little change in the placebo group (table 2).

Effect of supplementation on end point measures

A 16-week supplementation with vitamin D_3 (10 or 25 µg combined compared) to placebo had no significant effect on HbA1c, fructosamine, total cholesterol, LDL, HDL, and triglycerides or BMI (table 2).

Subgroup analyses

There were no significant effects on outcome variables in subgroups defined by baseline values (figure 2). There were also no significant effects of 25 µg vitamin D versus placebo on any of the end points (data not shown).

Furthermore, there were no significant differences between the combined intervention groups and placebo in any of the end points after stratification by baseline concentration of s-25(OH)D higher or lower than 25 nmol/L, or after stratification by gender (data not shown).

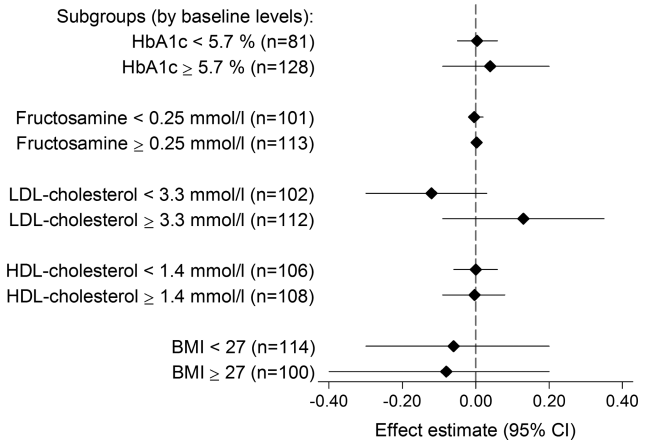

Figure 2 Subgroup analysis. Effect of vitamin D supplementation on outcome variables in subgroups defined by baseline levels of the respective outcome variables. Effect estimates for each outcome variable are difference in change from baseline to 16 weeks between the combined intervention groups (10 or 25 µg) and the placebo group. Within each subgroup, the effect estimate was adjusted for the baseline level of the respective outcome variables (baseline value entered as a continuous variable in the regression model).

DISCUSSION

Principal findings

In this study, we found that a 16-week supplementation with either 10 or 25 µg vitamin D_3 daily to healthy immigrants from South Asia, the Middle East, or Africa and now living in Norway did not significantly affect HbA1c, fructosamine, serum lipids, or BMI. Notably, the 95% CI for mean difference in HbA1c (−0.04 to 0.06) excluded −0.5%, which was our a priori defined smallest difference of clinical relevance. We can therefore reasonably exclude large effects on HbA1c of a 16-week intervention with these dosages.

Strengths and weaknesses

The study was a strictly performed double-blinded, randomized, placebo-controlled trial with good compliance and relatively high retention. According to our prestudy power calculation, our relatively large sample size should provide good statistical power to detect even small-to-moderate effects on HbA1c. Blood samples were assayed in one batch. In addition to the commonly measured HbA1c, we also included fructosamine, which refers to glycated serum proteins and reflects the average blood glucose levels for the previous 2–3 weeks. We reasoned that this would increase the chance of detecting changes during a 16-week intervention period. The ethnic minorities targeted in our study are known to have a generally poor vitamin D status and relatively high risk of type 2 diabetes and other cardiovascular risk factors, as confirmed in our study.[1 6 19 20] The study also has some limitations. It was designed primarily for muscular strength outcomes, but HbA1c was an important prespecified outcome described in the protocol. We did not measure fasting glucose levels and insulin sensitivity, which would have been more demanding for the participants with possibly negative effects on recruitment and participation of the study participants. Also, the participants may not have been exposed for a sufficiently long time to high levels of circulating s-25(OH)D to affect some of our outcome variables.

Findings in relation to other studies

A recently published systematic review and meta-analysis concluded that currently there is insufficient evidence to recommend vitamin D supplementation in order to improve glycemia or insulin resistance in patients with diabetes, normal fasting glucose, or impaired glucose tolerance.[21] In the systematic review, only four studies examined HbA1c as an outcome, and all were done in participants with diabetes or another serious chronic disease, or the intervention was done with 1-hydroxyvitamin D, which circumvents the strictly controlled hydroxylation of the 1-position (normally of 25(OH)D).

A few other relevant studies including healthy participants with low vitamin D status have been published after the systematic review cited above. Studies with similar characteristics as ours, with a duration of 3–12 months, consistently did not show clear effects on HbA1c (see online electronic supplementary material for details), and generally supported our result. For instance, Davidson *et al*[22] examined healthy persons from ethnic minorities in the USA (Latinos and African-Americans) with HbA1c >5.8% and s-25(OH)D <75 nmol/L at baseline. They showed no effect of high doses of vitamin D supplementation (doses corresponding to >200 µg/day) for 1 year on various measures of glycemia or insulin sensitivity. HbA1c was slightly reduced in those receiving vitamin D, but the effect size was deemed clinically non-relevant. While we did not restrict participants by baseline s-25(OH)D or HbA1c, the large majority of our participants in our study had levels <50 nmol/L and many had HbA1c >5.7%. Harris *et al*[23] gave a 3-month supplementation with 4000 IU (100 µg) vitamin D_3 daily in overweight African-Americans with prediabetes but found no effect on HbA1c or other measures of glycemia. A RCT by Mitri *et al*[24] examining the effect of vitamin D supplementation in adults at high risk of diabetes concluded that short-term supplementation with cholecalciferol did not have a significant effect on HbA1c.

In two other relatively large studies performed in the North of Norway, capsules of 20 000 IU (500 µg) vitamin D_3 or placebo were given twice weekly for 6 months to healthy Norwegians with s-25(OH)D levels <50 nmol/L, but the intervention did not improve HbA1c.[25] We did not identify any other randomized studies of the effect of vitamin D on fructosamine. The lack of effect on fructosamine is nevertheless consistent with the lack of effect on HbA1c and on fasting glucose seen in other studies[21] since fructosamine also reflects aspects of glucose metabolism.

Results from systematic reviews of RCTs of vitamin D on lipids show a lack of effect of vitamin D on total cholesterol, LDL-cholesterol, and HDL-cholesterol which was clearly consistent with our study.[13 26] Also, our updated meta-analysis of recent trials similar to ours supported the lack of effect of vitamin D on serum total cholesterol in healthy adults (confer electronic supplementary material). A consistent lack of effect of vitamin D on serum lipids was also found in a 12-month study among Pakistani immigrants in Copenhagen, Denmark.[27]

A recently conducted review of RCTs on either vitamin D plus calcium or only vitamin D, and reporting effects on adiposity outcomes including BMI, concluded that current evidence from RCTs did not consistently support the contention that calcium and vitamin D accelerated weight or fat loss in obesity.[28] Also, von Hurst *et al* conducted a randomized controlled study in 81 women of South Asian origin living in New Zealand and aged 23–68 years to 6 months of supplementation with 100 µg/day of vitamin D or placebo and stated that there was no significant effect on BMI. In accordance with other RCTs, we did not find any effect of vitamin D supplementation on BMI.[29 30] Our updated meta-analysis on BMI clearly showed that there was no

indication on an effect of vitamin D on BMI in healthy adults (see online electronic supplementary material).

In our study, both doses of vitamin D supplementation were sufficient to raise s-25(OH)D concentration significantly compared with the placebo group, but ≥50 nmol/L was not reached in 43% (25 µg supplementation group) and 62% (10 µg supplementation group).

The existing literature on the effect of vitamin D on the end points included in our study did not identify any effects even when higher doses (even above 100 µg/day) and longer duration (up to 12 months) of treatment with vitamin D were used. Thus, we believe that the lack of effect in our study would not change with another regime of vitamin D treatment.

In conclusion, in healthy adults with an immigrant background low in vitamin D, supplementation with vitamin D_3 during 16 weeks did not improve HbA1c, fructosamine, lipid profiles, and BMI. Our literature review and updated meta-analysis showed that the lack of effect of vitamin D on these end points in healthy adults was consistent and corroborated by other similar studies.

Future research

Over 1/3 of the study participants had prediabetes HbA1c levels and are at risk of development of diabetes; therefore, exploring effective preventive means for vitamin D deficiency, diabetes, and cardiovascular disease is urgently needed. Also, more work is needed to study the long-term health consequences of the low vitamin D status of immigrants.

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Acknowledgements The authors express their gratitude to all study participants. Thanks to all those organizations, such as KIA center, and other centers which allowed us to use their venues for recruitment and data collection. Thanks to Marie Buchmann and Anne-Lise Sund at the Fürst Medical Laboratory for their invaluable assistance and expert advice on blood collection and analysis, Eva Kristensen for her assistance with data collection, Ingvild Dalen and Magne Thoresen for statistical consultation. Finally, thanks to Anne Karen Jenum for the biobank support.

Contributors AAM and KVK designed the study protocol and conducted and collected the data. LCS conducted a literature search, summary of the literature, and updated the meta-analysis with input from AAM, KVK, and HEM. AAM carried out the analysis and drafted the manuscript. KVK, LCS, HEM, MB, and PL contributed to the planning and design of the study and the interpretation of data, as well as a critical revision of the manuscript. All authors approved the final manuscript to be submitted. AAM and HEM are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Funding This research was funded by the Norwegian Women's Public Health Association and University of Oslo. The study was also supported by the Fürst Medical Laboratory and by Nycomed Pharma AS, which included free trial drugs.

Competing interests None.

Ethics approval The study was approved by the Regional Committee for Medical and Health Research Ethics (study code: 2010/1982).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

1. Madar AA, Stene LC, Meyer HE. Vitamin D status among immigrant mothers from Pakistan, Turkey and Somalia and their infants attending child health clinics in Norway. *Br J Nutr* 2009;101:1052–8.
2. Holvik K, Meyer HE, Haug E, *et al*. Prevalence and predictors of vitamin D deficiency in five immigrant groups living in Oslo, Norway: the Oslo Immigrant Health Study. *Eur J Clin Nutr* 2005;59:57–63.
3. Andersen R, Molgaard C, Skovgaard LT, *et al*. Pakistani immigrant children and adults in Denmark have severely low vitamin D status. *Eur J Clin Nutr* 2008;62:625–34.
4. Lips P. Vitamin D status and nutrition in Europe and Asia. *J Steroid Biochem Mol Biol* 2007;103:620–5.
5. Bouillon R, Carmeliet G, Verlinden L, *et al*. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev* 2008;29:726–76.
6. Jenum AK, Diep LM, Holmboe-Ottesen G, *et al*. Diabetes susceptibility in ethnic minority groups from Turkey, Vietnam, Sri Lanka and Pakistan compared with Norwegians—the association with adiposity is strongest for ethnic minority women. *BMC Public Health* 2012;12:150.
7. Wandell PE, Carlsson A, Steiner KH. Prevalence of diabetes among immigrants in the Nordic countries. *Curr Diabetes Rev* 2010;6:126–33.
8. Barnett AH, Dixon AN, Bellary S, *et al*. Type 2 diabetes and cardiovascular risk in the UK south Asian community. *Diabetologia* 2006;49:2234–46.
9. Tran AT, Straand J, Diep LM, *et al*. Cardiovascular disease by diabetes status in five ethnic minority groups compared to ethnic Norwegians. *BMC Public Health* 2011;11:554.
10. Gholap N, Davies M, Patel K, *et al*. Type 2 diabetes and cardiovascular disease in South Asians. *Prim Care Diabetes* 2011;5:45–56.
11. Pittas AG, Nelson J, Mitri J, *et al*. Plasma 25-hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. *Diabetes Care* 2012;35:565–73.
12. Hutchinson MS, Figenschau Y, Njolstad I, *et al*. Serum 25-hydroxyvitamin D levels are inversely associated with glycated haemoglobin (HbA(1c)). The Tromso Study. *Scand J Clin Lab Invest* 2011;71:399–406.
13. Jorde R, Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res* 2011;50:303–12.
14. Wang TJ, Pencina MJ, Booth SL, *et al*. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008;117:503–11.
15. Knutsen KV, Madar AA, Lagerlov P, *et al*. Does vitamin D improve muscle strength in adults? A randomized, double-blind, placebo-controlled trial among ethnic minorities in Norway. *J Clin Endocrinol Metab* 2014;99:194–202.
16. Nathan DM, Kuenen J, Borg R, *et al*. Translating the A1C assay into estimated average glucose values. *Diabetes Care* 2008;31:1473–8.
17. Wright LAC, Hirsch IB. The Challenge of the use of glycemic biomarkers in diabetes: reflecting on hemoglobin A1C, 1,5-anhydroglucitol, and the glycated proteins fructosamine and glycated albumin. *Diabetes Spectr* 2012;25:141–8.
18. Jorde R, Figenschau Y. Supplementation with cholecalciferol does not improve glycaemic control in diabetic subjects with normal serum 25-hydroxyvitamin D levels. *Eur J Nutr* 2009;48:349–54.
19. Hjellset VT, Bjorge B, Eriksen HR, *et al*. Risk factors for type 2 diabetes among female Pakistani immigrants: the InvaDiab-DEPLAN study on Pakistani immigrant women living in Oslo, Norway. *J Immigr Minor Health* 2011;13:101–10.

20. Knutsen KV, Brekke M, Gjelstad S, *et al*. Vitamin D status in patients with musculoskeletal pain, fatigue and headache: a cross-sectional descriptive study in a multi-ethnic general practice in Norway. *Scand J Prim Health Care* 2010;28:166–71.
21. George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabet Med* 2012;29: e142–50.
22. Davidson MB, Duran P, Lee ML, *et al*. High-dose vitamin d supplementation in people with prediabetes and hypovitaminosis d. *Diabetes Care* 2013;36:260–6.
23. Harris SS, Pittas AG, Palermo NJ. A randomized, placebo-controlled trial of vitamin D supplementation to improve glycaemia in overweight and obese African Americans. *Diabetes Obes Metab* 2012;14:789–94.
24. Mitri J, Dawson-Hughes B, Hu FB, *et al*. Effects of vitamin D and calcium supplementation on pancreatic beta cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *Am J Clin Nutr* 2011;94:486–94.
25. Jorde R, Strand Hutchinson M, Kjaergaard M, *et al*. Supplementation with high doses of vitamin D to subjects without vitamin D deficiency may have negative effects: pooled data from four intervention trials in Tromso. *ISRN Endocrinol* 2013;2013:348705.
26. Elamin MB, Abu Elnour NO, Elamin KB, *et al*. Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 2011;96:1931–42.
27. Andersen R, Brot C, Mejborn H, *et al*. Vitamin D supplementation does not affect serum lipids and lipoproteins in Pakistani immigrants. *Eur J Clin Nutr* 2009;63:1150–3.
28. Soares MJ, Chan She Ping-Delfos W, Ghanbari MH. Calcium and vitamin D for obesity: a review of randomized controlled trials. *Eur J Clin Nutr* 2011;65:994–1004.
29. Wamberg L, Kampmann U, Stodkilde-Jorgensen H, *et al*. Effects of vitamin D supplementation on body fat accumulation, inflammation, and metabolic risk factors in obese adults with low vitamin D levels —results from a randomized trial. *Eur J Intern Med* 2013;24:644–9.
30. Sneve M, Figenschau Y, Jorde R. Supplementation with cholecalciferol does not result in weight reduction in overweight and obese subjects. *Eur J Endocrinol* 2008;159:675–84.

Supplementary material:
Literature search, summary of literature, and updated meta-analysis

Literature search strategy

Literature searches were repeated several times from the time of drafting of the protocol and during the study, with a final, updated search on October 30, 2013. The strategy was to first identify recent published systematic reviews of randomized placebo controlled clinical trials of vitamin D supplementation and the response-variables studied in this paper (i.HbA1c or fructosamine, ii. serum cholesterol and iii. body mass index) using search terms specified below. Then, to identify original studies on HbA1c published between the period covered by the systematic reviews and October 30, 2013, we repeated the search without restriction to systematic reviews (but adding a restriction to randomised trials, see search terms below). Titles and abstracts identified were screened by LCS to identify studies of direct relevance for comparison with our results. In addition, we (AA, KVK, HE, LCS) performed additional informal searches of PubMed and other sources, and we inspected reference lists in papers of specific interest to potentially identify further publications of relevance.

The *search terms* used in the search for systematic reviews were as follows:

1. ("systematic review" OR “meta analysis” OR "meta-analysis") AND ("vitamin D" OR cholecalciferol) AND (HbA1c OR A1C OR glycaemia OR glycemia OR glucose OR “glycated haemoglobin” OR “glycated hemoglobin” OR fructosamine)
2. ("systematic review" OR “meta analysis” OR "meta-analysis") AND ("vitamin D" OR cholecalciferol) AND (lipid* OR cholesterol)
3. ("systematic review" OR “meta analysis” OR "meta-analysis") AND ("vitamin D" OR cholecalciferol) AND (BMI OR “body mass index” OR obesity OR adiposity)

Criteria for studies to be of direct relevance to ours

Studies were considered relevant for direct comparison and potential inclusion in an informal meta-analysis together with our results if they met the following criteria:

The design should be randomized controlled trials (RCTs) with a placebo group, and reporting dependent variables included in our study measured at baseline and after a follow-up of at least 12 weeks (HbA1c, fructosamine, serum total cholesterol, serum low-density lipoprotein (LDL-) cholesterol, or serum high-density lipoprotein (HDL-) cholesterol, or body mass index (BMI)).

Intervention should be oral supplementation with vitamin D (vitamin D3 or D2, *not* 1-hydroxyvitamin D or 1,25-hydroxyvitamin D) as the only intervention.

Subjects should be non-pregnant participants aged 18 or above without a diagnosis of diabetes or other serious, chronic disease (studies in subjects with obesity or “prediabetes” that are normally not considered diagnoses were included, and relevant studies in other groups were considered for discussion but not for inclusion in meta-analysis).

Methods of summarising data: Results were summarised as estimated mean differences [(mean in treatment group at follow up – treatment group at baseline –(placebo group at follow up – placebo group at baseline)] with 95% confidence intervals. Standard deviations of mean change in each group were extracted from the papers, and if not reported (specified in the text below) we imputed this by calculating the weighted mean variance from the studies with available estimates as described by Follmann *et al.*(1) These were plotted using metan in Stata, version 12. Estimates were pooled as the weighted mean difference (fixed effects meta-analysis).

HbA1c or fructosamine

Search 1 gave 11 hits on October 30, 2013, of which the following George *et al*(2), Thomas *et al*(3) and Mitri *et al*(4) were most updated and relevant. Search in the Cochrane database did not provide any additional relevant reviews. The informal search led to the identification of a systematic review by Autier et al. published online Dec 6, 2013, and finally published in the January 2014 issue of the new journal Lancet Diabetes & Endocrinology, which is not yet indexed in PubMed; http://www.thelancet.com/journals/landia/article/PIIS2213-8587(13)70165-7/fulltext (5). This systematic review searched for both observational cohort studies and randomised controlled trials of vitamin D and a variety of biomarker and health outcomes in PubMed and Embase up to Dec 31, 2012. The results of this paper were identified after our main search and updated meta-analysis, and are briefly commented on below).

George *et al*(2) searched for publications up to March 2011 and identified 4 studies reporting HbA1c and no trials with fructosamine as the endpoint. From a total of 15 studies reporting on one or more of these endpoints, the main conclusion was that "no significant improvement was seen in fasting glucose, HbA1c or insulin resistance in those treated with vitamin D compared with placebo". Many studies were done in subjects with a diagnosis of diabetes at baseline, and of the four studies with HbA1c, this was the case for three. One study reporting on HbA1c was done with 1-hydroxy-vitamin D or placebo to subjects with prediabetes. Therefore no study was strictly comparable to ours.

Thomas *et al*(3) aimed to search for "clinical studies evaluating the impact of vitamin D on aspects of hyperglycaemia in non-pregnant adults and searched PubMed for publications during 1950 - May 2011. In the abstract, the authors concluded that "No well-designed randomised, controlled trials were identified that specifically investigated the effects of vitamin D supplementation on glucose and insulin concentrations".

Mitri *et al*(4) performed a "systematic review of longitudinal observational studies of vitamin D status and trials of vitamin D supplementation on glycemic outcomes". They searched MEDLINE and the Cochrane Database of Systematic Reviews through February 2011. They reported in the abstract that "In post hoc analyses from eight trials among participants with normal glucose tolerance at baseline and in three small underpowered (n 32–62) trials of patients with established type 2 diabetes, there was no effect of vitamin D supplementation on glycemic outcomes. In two trials among patients with baseline glucose intolerance, vitamin D supplementation improved insulin resistance."

Collectively, these 3 systematic reviews identified 4 trials reporting results on HbA1c, all of which were included in George et al. and none of which were comparable to our study. No trials were identified that reported results of fructosamine as the outcome. Few of the studies reported were done in African, Asians, or ethnic minorities or other groups know to have poor vitamin D status at baseline.

Search for recent original trials of HbA1c or fructosamine

The search for recent original trials (using the terms ("vitamin D" OR cholecalciferol) AND (HbA1c OR A1C OR glycaemia OR glycemia OR glucose OR "glycated haemoglobin" OR "glycated hemoglobin" OR fructosamine) AND (random* OR intervention OR trial) AND ("2011/01/01"[Date - Publication] : "2013/11/30"[Date - Publication])), identified 129 publications. Of these, the following five were judged to be of direct relevance to our study results and reported results on HbA1c (detailed in Supplemental Table 1): (6-10).

Another two original trials reported on other measures of glycaemia such as fasting glucose, glucose 2 hours after an oral glucose tolerance test (11,12). Gepner *et al*(11) randomized 114 healthy, community-dwelling post-menopausal women from Madison, Wisconsin, with 25OHD concentrations >10 and <60 ng/mL (mean 31 ng/mL) at baseline to 2500 IU (62.5 µg) vitamin D_3 or placebo, daily for 4 months. No significant effect was observed on fasting glucose. Muldowney *et al*(12). Conducted two RCTs of 5, 10, or 15 μg/d of vitamin D vs placebo during winter time (22 week duration). Subjects were healthy, white skinned men and women in the south and north of Ireland. One study included 202 subjects aged 20–40 years and the other included 192 subjects aged ≥64 years. Except for the group who received 15 μg vitamin D/d, there was little increase in 25OHD after intervention (but a drop in the placebo group). 25(OH)D decreased from baseline to endpoint, except in the 15 μg/d group, who maintained the baseline concentration of ~70 nmol/L. No significant effect was seen on glucose or other endpoints (lipids results discussed below).

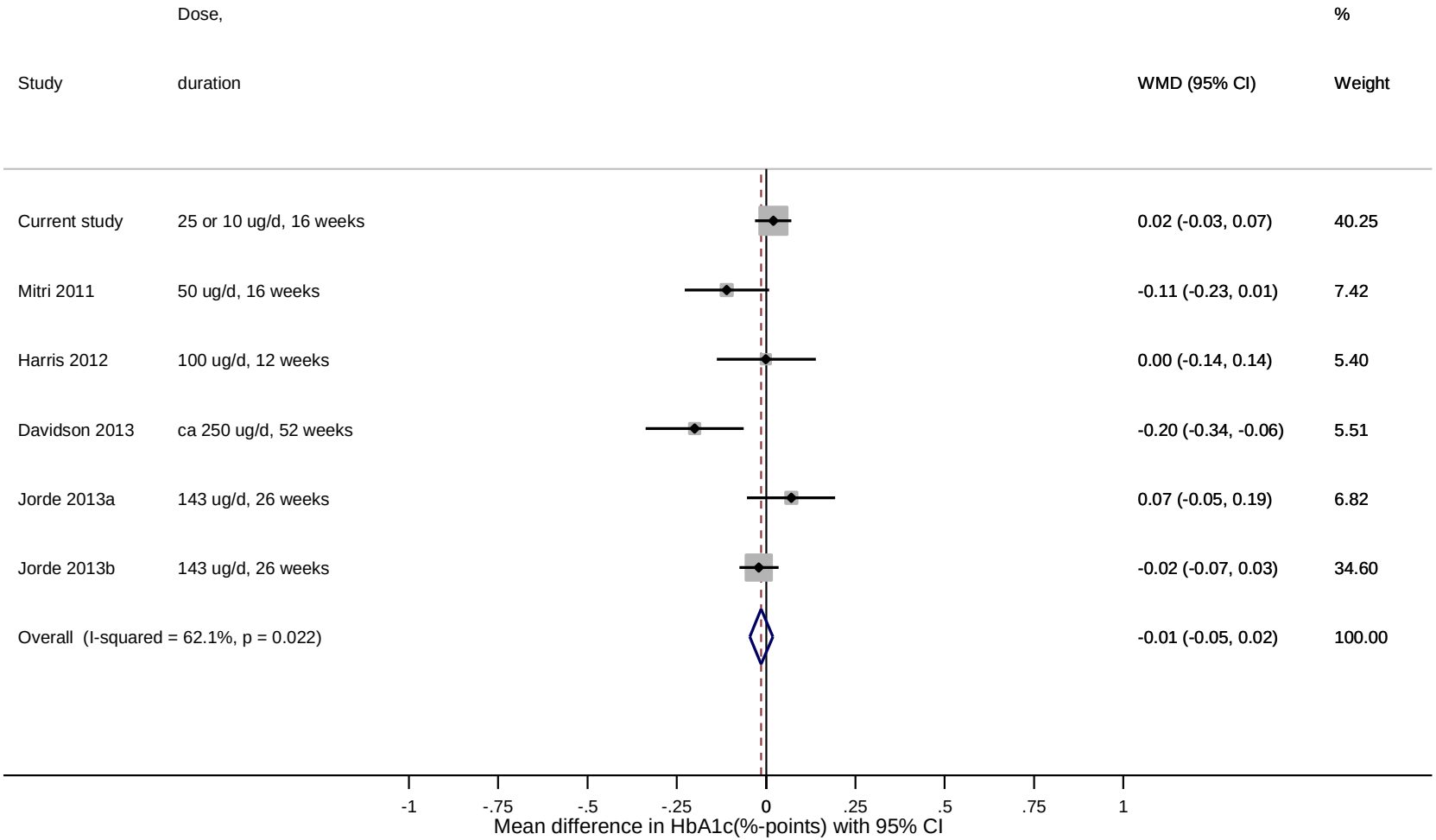
The recently published review by Autier *et al* (5) identified 16 randomised trials of vitamin D supplements reporting the effect on HbA1c, of which only six were in subject without diagnosed diabetes. Their meta-analysis showed a remarkably consistent lack of effect, even if the design and target groups were quite heterogeneous. Our search (above) and inclusion of studies considered relevant to our updated meta-analysis (below) identified all studies of relevance among those covered in Autier *et al*.

Supplemental Table 1: Recent randomised trials of oral vitamin D supplementation for at least eight weeks, reporting results on change in HbA1c in populations without a diagnosis of diabetes or other serious chronic disease.

Study	Country	Inclusion criteria	Intervention	Sample size (intervention + placebo)	Duration	Relevant endpoint reported*	Main result	Ref
Current study	Norway	Immigrants from Africa, Middle East or Asia, age 18-50y	25 or 10 µg vit D_3/d vs placebo	(75+69) + 71	16 weeks	HbA1c, fructosamine	HbA1c mean change from baseline was 0.03 %-points in the intervention group (SD:0.17) vs 0.01 (SD:0.18) for placebo; effect estimate adjusted for baseline HbA1c: 0.02 %-points (95%CI: -0.04, 0.06)	
Mitri 2011	USA	>40y, BMI 25-40 with glucose intolerance but no diabetes diagnosis (mean ~60 nM at baseline)	50 µg vit D/d vs placebo‡‡	22 + 22‡‡	16 weeks	HbA1c, FPG, 2h PG, + other measures	HbA1c changed by 0.07 in the vitamin D group and 0.18 in the placebo group (p(diff): 0.07. Significant beneficial effect on FPG, and on beta-cell function (primary outcome).	(6)
Harris 2012	USA	African Americans age >=40 y (mean ~57) BMI 25-39.9, "prediabetes"§ no diagnosis of diabetes and otherwise healthy (mean 25OHD ≈39 nM at baseline)	4,000 IU vit D_3/d (=100 µg/d) vs placebo	43+46	12 weeks	HbA1c, FPG, 2h PG	No significant effect; mean change in HbA1c in intervention group was -0.05 %-points (SEM: 0.05) vs -0.05 %-points in the placebo (SEM:0.05); effect estimate=0.00. For fasting glucose the corresponding effect estimate was +0.02 mM, and for 2h post-load glucose: +0.01 mM	(7)
Salehpour 2013	Iran	Healthy overweight or obese, premenopausal women age 18-50y (mean 25OHD 37 nM and 47 nM in the intervention and placebo groups, respectively, at baseline)	25 µg vit D_3/d vs placebo	39 + 38	12 weeks (90 d)	HbA1c, FPG, 2h PG	No statically significant effect on either outcome (HbA1c change: -1 (SD:0.5) in intervention group vs. -0.4 (SD:0.6) in placebo; effect estimate=-0.6%-points; For glucose: -0.28 – (-0.65)=0.37 mM; for 2 h post load glucose: -0.29-(-0.30)=0.01 mM. Inconsistent reporting of HbA1c results, see text.	(8)
Jorde 2013a (insulin sensitivity study) ††	Norway	Age 30-75y, 25OHD <50nM (mean 40.8 nM (SD:13.1) at baseline)	20,000 IU vit D twice per week (≈143 µg/d) vs placebo	49+45	26 weeks	HbA1c	+0.12%-points (SD: 0.32) for intervention vs. 0.05 (SD=0.29) for placebo; effect estimate (difference): 0.12-0.05=0.07%-points	(9)
Jorde 2013b ("depression study") ††	Norway	Age 30-75y, 25OHD <55nM (mean 47.5nM (SD: 15.6) at baseline)	20,000 IU twice per week (≈143 µg/d) vs placebo	119+109	26 weeks	HbA1c	-0.03% (SD: 0.20) vs. placebo: -0.01% (SD 0.22); effect estimate (difference) = =-0.02%-points	(9)
Davidson 2013	USA	Latino (~90%) or African Americans aged >=40y with prediabetes† and 25OHD <75nM (mean 25OHD was ~55nM at baseline)	Approx . 200-300 µg vit D/d **	~49 + ~49	52 weeks	HbA1c, FPG, 2h PG	HbA1c changed from 6.1 to 6.0% vs from 6.1 to 6.2% for placebo, resulting in a significant effect estimate of -0.2%-points (SD not provided, other than SD of mean at baseline: 0.4 in placebo and 0.3 in intervention); no significant effect on other measures of glycaemia	(10)

* FPG: fasting plasma glucose; 2h PG: glucose 2 hours after oral glucose tolerance test. † Complex inclusion criteria: Subjects without a diabetes diagnosis, but with at least one risk factor (increased waist circumference, family history of diabetes or history of gestational diabetes or hypertension) were screened first with HbA1c after which those with values 5.9-6.9 % underwent oral glucose tolerance test to identify subjects with prediabetes, defined as fasting plasma glucose 110–125 mg/dl or 2-h post load glucose value of 140–199 mg/dL. ‡ The numbers randomised to 5,10 and 15 µg vitamin D/d, respectively in parenthesis. § About 25% were diabetic if defined by baseline HbA1c and/or fasting glucose criteria alone. ‖ To convert glucose from mg/dl to µmol/l (mM), multiply by 0.0555. ** Complex dosing scheme calculated as (80 or 100 minus baseline serum 25OHD in ng/ml) x kg body weight x 15.7 = IU/week. For 70 kg person with baseline 25OHD of 55 nM (=22 ng/ml) this would be 63,742 or 85,722 IU/week ≈ 228-306 µg vit D/d . †† The "insulin sensitivity study" was originally published (including HbA1c) in Grimnes et al. Diabetes 2011;60:2748-2757 (including HbA1c results), while the "depression study" was originally reported by Kjærgaard et al. Br J Psychiatry 2012, but without HbA1c results. 25OHD: 25-hydroxyvitamin D. ‡‡ We excluded participants receiving calcium from our analysis to comply with our inclusion criteria.

Supplemental Figure 1. Effect of vitamin D on HbA1c in healthy adults (total n=764), data from Supplemental table 1. WMD: Weighted mean difference. Doses given are for oral vitamin D supplements, and WMD is difference from placebo group.



The results for HbA1c in Jorde *et al* (9) (two trials) and Harris et al (7) were similar to ours, while Davidson *et al*'s study (10) with extremely high doses suggested a significant beneficial effect of a magnitude that was deemed a no or little clinical significance. Mitri *et al*'s study suggested a borderline significant beneficial effect of vitamin D on HbA1c, but note that the absolute HbA1c increased in both groups (less so in the intervention group) during the 16 week intervention. Salehpour *et al* (8) reported data on HbA1c that was partially inconsistent. The point estimate of effect was -0.6%-points, which is stronger than all other studies. The authors reported a p-value of 0.06 (not significant), but this was not consistent with standard deviations and mean estimates in their table, and we decided not to include their data in the final meta-analysis. Inclusion of Salehpour *et al*'s data would lead to more marked heterogeneity between studies, but the pooled estimate was little changed (-0.02, 95%CI: -0.05, 0.02). We contacted the authors but we were unfortunately not able to obtain consistent estimates allowing us to confidently include the data in the meta-analysis. While the test for heterogeneity was statistically significant, a the magnitude of effects estimated in the different studies were judged to be of very limited clinical relevance in all cases. We also run a sensitivity analysis by employing a random effects model. This resulted in relatively smaller weights for the two large studies (the current and Jorde 2013b), but the weighted mean difference estimate was essentially unchanged (-0.03, 95% CI: -0.09, 0.03). Despite some differences in inclusion criteria between the recently published meta-analysis by Autier et al. (5) and ours, the main result was comparable except an apparently lesser degree of statistical heterogeneity in their analysis. This seems paradoxical because we made an attempt to include studies with similar design and target groups. Among 23 trials of vitamin D supplements and the effect of fasting plasma glucose identified by Autier et al (5), all but one were not statistically significant but no formal meta-analysis with forrest plot were presented. In summary, the available evidence suggest no significant effect of vitamin D on HbA1c in adults with low levels of 25OHD but otherwise without diagnoses of chronic diseases. Any potential effect is likely to very small.

Serum lipids

Search 2 gave 13 hits in PubMed on October 30, 2013, and the following two were the most updated and relevant: (13,14). A search in the Cochrane database did not provide any additional relevant reviews.

Elamin *et al* (13) systematically reviewed the literature for randomised controlled trials of vitamin D up to the end of August 2010. The review was primarily concerned with death and cardiovascular diseases as the endpoint, but included lipids and glucose as a secondary outcome. Among a total of 51 trials with moderate quality, they pooled results from 12 trials reporting on serum lipids. The weighted mean difference was 0.00 (95% CI: -0.06, 0.07) for total cholesterol (12 studies), -0.09 (95% CI: -0.24, 0.07) for LDL-cholesterol (11 studies), and 0.06 (95%CI: -0.11, 0.24) for HLD-cholesterol (12 studies). The I-squared as an estimate of between study heterogeneity was 28, 90, and 99% for total-, LDL-, and HDL-cholesterol, respectively.

Wang *et al* (14) systematically searched the literature up to the end of October 2011. They included in the meta-analysis 10-11 RCTs (7-11 studies, depending on lipid fraction), all with differences in design or inclusion criteria from our study (for instance including patients with diagnoses such as type 2 diabetes, interventions in addition to vitamin D such as weight loss or calcium supplement, and including 1-hydroxyvitamin D supplements). In their table 3 they reported a weighted mean difference in total cholesterol that significantly increased in those who received vitamin D supplements (3.23 mg/dl, 95%CI: 0.55, 5.90; or 0.08 mmol/l (95% CI: 0.01, 0.15)). However this result was referred to in the text and abstract as if it was LDL-cholesterol. Our attempt to contact the corresponding author by email to clarify this inconsistency was not successful.

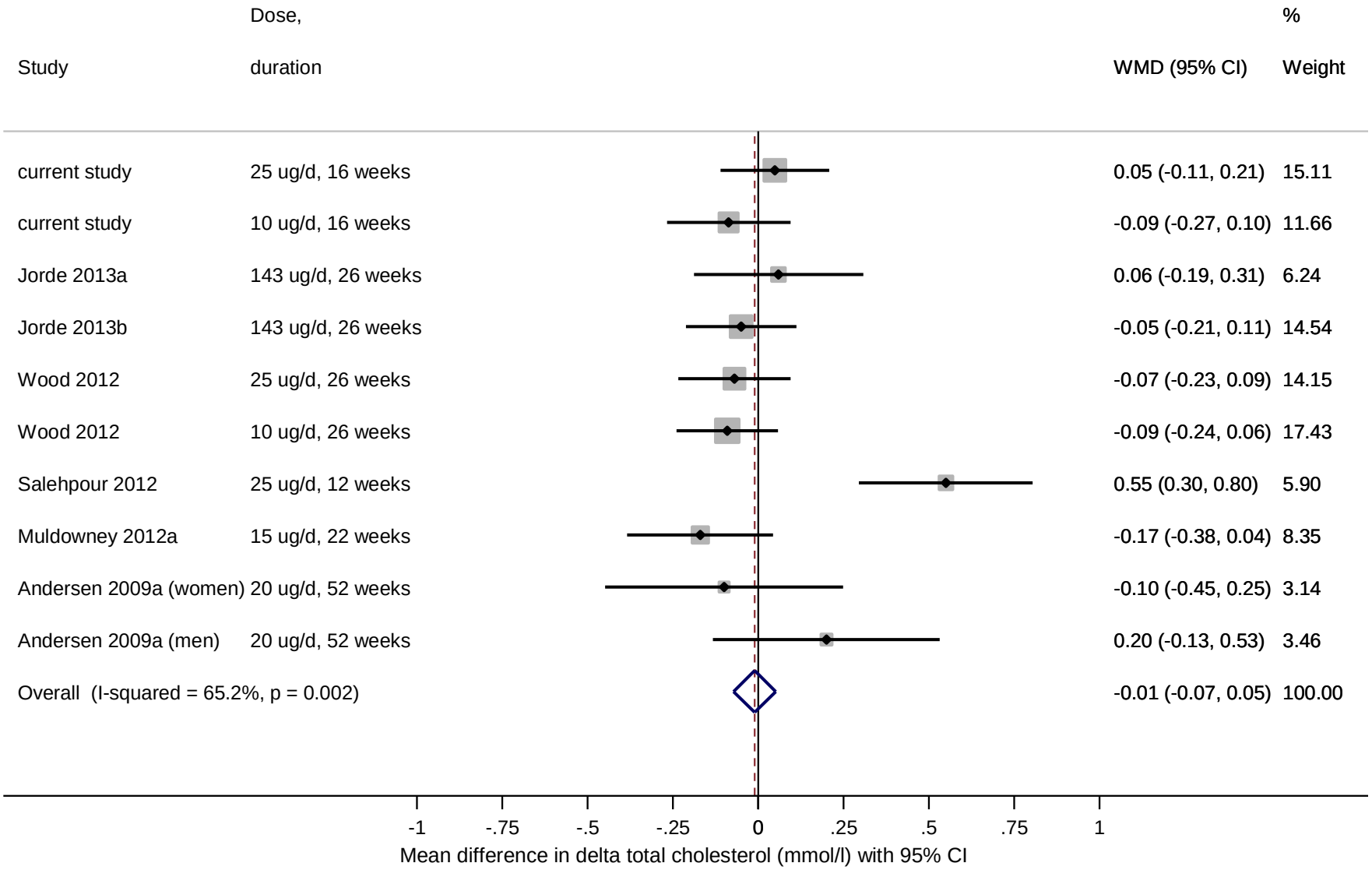
If assuming that results in the figures were correct, the pooled effect estimates showed a slightly but significantly increased total cholesterol in those who received vitamin D supplements, and no significant effect on LDL- or HDL-cholesterol.

Results from the meta-analyses conducted by Autier *et al* (5) concluded that nearly all trials, vitamin D supplementation did not affect concentrations of blood lipids (total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides).

Several of the studies who reported on HbA1c or glucose also showed data for lipids, and we informally summarised result for total cholesterol from these and other studies(15,16) with similar design to ours, and Salehpour et al reported results from serum lipids in a separate publication in 2012 (17) (Supplemental Figure 2). Note that Andersen et al did not report mean difference between follow-up and baseline, only median levels at each point, including in the baseline data those who were lost to follow-up at 12 months (about 26%). They also

included a group randomized to 10 µg vitamin D/d not included in the meta-analysis because the data reported were not strictly comparable to those from the other studies. We contacted the authors but they were unfortunately not able to provide the comparable estimates at present. Salehpour *et al*'s results(17) were different from the other studies in that they found significantly increased total cholesterol after vitamin D intervention. In summary, the lack of effect of vitamin D on total cholesterol in our study was clearly consistent with these other studies. Any potential effect would be very weak. The other studies also reported results on LDL-cholesterol and HDL-cholesterol and similarly did not find any significant effect of vitamin D, with the exception of Salehpour *et al* (17) who found significant increase in both LDL-cholesterol and HDL-cholesterol (data not shown).

Supplemental Figure 2. Effect of vitamin D on serum total cholesterol in healthy adults (total n=1199). WMD: Weighted mean difference.



Body mass index (BMI)

Search 3 gave 34 hits in PubMed on October 30, 2013. None of the identified studies were systematic reviews of randomized trials of effect of vitamin D supplementation on BMI, but we assessed Renzaho *et al* and Saneei *et al*, both of which focussed on observational studies(18,19). They both concluded that there is a need for randomized controlled trials, and from their reference lists we identified a review of RCTs by Soares *et al*(20) as relevant for our purpose. A search in the Cochrane database did not provide any additional relevant reviews.

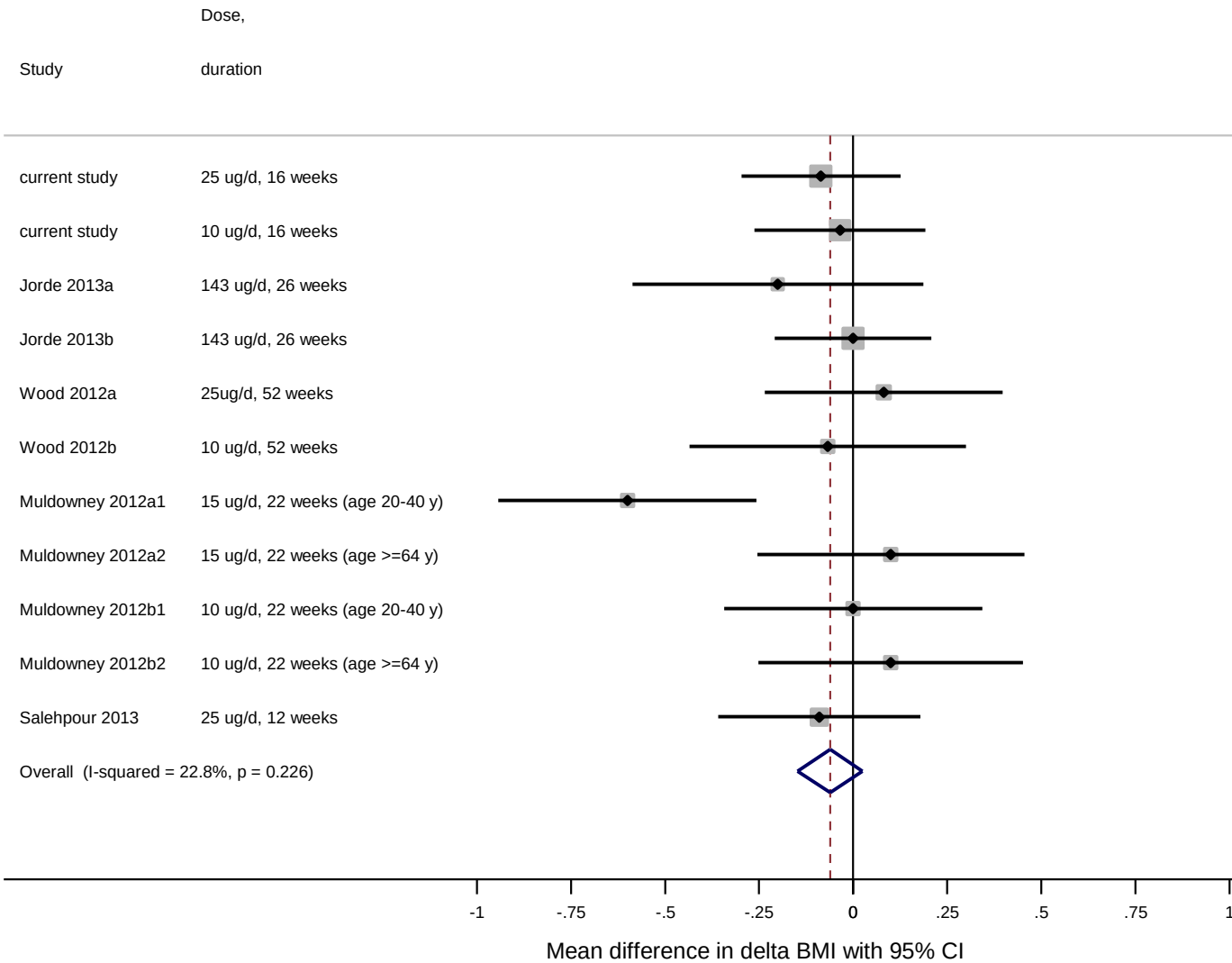
Soares *et al*(20) reviewed the literature from year 2000 to early 2011 in several databases, and focused primarily on RCTs randomizing participants to either vitamin D plus calcium or only vitamin D, and reporting effects on adiposity outcomes including BMI. The primary focus was effects during treatment of obesity (which is in contrast to our focus). Soares *et al* identified 15 trials for their analysis, of which 7 gave vitamin D alone. They concluded that "Current evidence from RCTs did not consistently support the contention that calcium and vitamin D accelerated weight or fat loss in obesity".

Among the trials reviewed by Soares *et al*, only one conformed to the strict criteria we outlined above (in the section on HbA1c) for comparability to our study, namely von Hurst *et al* (21). They randomised 81 women of South Asian origin living in New Zealand aged 23-68 years to 6 months of supplementation with 100 µg/d of vitamin D or placebo. Inclusion criteria were serum 25OHD <50nM and insulin resistance (defined as homeostasis model assessment 1 >1.93, which is approx. the upper quartile in this population) and/or triacylglycerol:HDL-cholesterol ratio >=3. Data on BMI was not shown, but the authors stated that there was no significant effect on BMI.

The 12 trials which include in systematic review by Autier *et al* (5), only results of the Women's Health Initiative (WHI) study showed significant, but small, weight loss associated with supplementation (mean loss of 0.13 kg, 95% CI 0.05–0.21).

In addition, most of the newer trials identified for HbA1c or glucose or serum lipids (discussed above) also included data on BMI. Unpublished data on BMI from Wood et al (16) were kindly obtained from H.E. MacDonald, University of Aberdeen. We informally summarised these together with our own results (Supplemental Figure 3). With the exception of one subgroup in Muldowney *et al* (12) and the overall results from other studies were quite consistent with our results suggesting no effect on BMI. Note that in Muldowney et al, the ANOVA test for variation in mean across four groups (placebo, 5 µg/d, 10 µg/d and 15 µg/d) was not significant (p=0.32) (12). It seems likely that any potential effect would be of small magnitude.

Supplemental Figure 3. Effect of vitamin D supplements on BMI in generally healthy adults. Studies with two doses were included with the same control (placebo) group twice and is therefore not strictly independent (total n=1293). Standard deviations for Muldowney were imputed as described in the text. WMD=Weighted mean difference compared to placebo.



REFERENCES

1. Follmann D, Elliott P, Suh I, Cutler J: Variance imputation for overviews of clinical trials with continuous response. *J Clin Epidemiol* 45:769-773, 1992
2. George PS, Pearson ER, Witham MD: Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. *Diabet Med* 29:e142-e150, 2012
3. Thomas GN, Scragg R, Jiang CQ, Chan W, Marz W, Pilz S, Kim HC, Tomlinson B, Bosch J, Lam TH, Cheung BM, Cheng KK: Hyperglycaemia and vitamin D: a systematic overview. *Curr Diabetes Rev* 8:18-31, 2012
4. Mitri J, Muraru MD, Pittas AG: Vitamin D and type 2 diabetes: a systematic review. *Eur J Clin Nutr* 65:1005-1015, 2011
5. Autier P, Boniol M, Pizot C, Mullie P: Vitamin D status and ill health: a systematic review. *The Lancet Diabetes & Endocrinology* 2:76-89, 2014
6. Mitri J, Dawson-Hughes B, Hu FB, Pittas AG: Effects of vitamin D and calcium supplementation on pancreatic beta cell function, insulin sensitivity, and glycemia in adults at high risk of diabetes: the Calcium and Vitamin D for Diabetes Mellitus (CaDDM) randomized controlled trial. *Am J Clin Nutr* 94:486-494, 2011
7. Harris SS, Pittas AG, Palermo NJ: A randomized, placebo-controlled trial of vitamin D supplementation to improve glycaemia in overweight and obese African Americans. *Diabetes Obes Metab* 14:789-794, 2012
8. Salehpour A, Shidfar F, Hosseinpanah F, Vafa M, Razaghi M, Amiri F: Does vitamin D3 supplementation improve glucose homeostasis in overweight or obese women? A double-blind, randomized, placebo-controlled clinical trial. *Diabet Med* 30:1477-1481, 2013
9. Jorde R, Strand HM, Kjaergaard M, Sneve M, Grimnes G: Supplementation with High Doses of Vitamin D to Subjects without Vitamin D Deficiency May Have Negative Effects: Pooled Data from Four Intervention Trials in Tromso. *ISRN Endocrinol* 2013:348705, 2013
10. Davidson MB, Duran P, Lee ML, Friedman TC: High-dose vitamin D supplementation in people with prediabetes and hypovitaminosis D. *Diabetes Care* 36:260-266, 2013

11. Gepner AD, Ramamurthy R, Krueger DC, Korcarz CE, Binkley N, Stein JH: A prospective randomized controlled trial of the effects of vitamin D supplementation on cardiovascular disease risk. *PLoS ONE* 7:e36617, 2012
12. Muldowney S, Lucey AJ, Hill TR, Seamans KM, Taylor N, Wallace JM, Horigan G, Barnes MS, Bonham MP, Duffy EM, Strain JJ, Cashman KD, Kiely M: Incremental cholecalciferol supplementation up to 15 mug/d throughout winter at 51-55 degrees N has no effect on biomarkers of cardiovascular risk in healthy young and older adults. *J Nutr* 142:1519-1525, 2012
13. Elamin MB, Abu Elnour NO, Elamin KB, Fatourechi MM, Alkatib AA, Almandoz JP, Liu H, Lane MA, Mullan RJ, Hazem A, Erwin PJ, Hensrud DD, Murad MH, Montori VM: Vitamin D and cardiovascular outcomes: a systematic review and meta-analysis. *J Clin Endocrinol Metab* 96:1931-1942, 2011
14. Wang H, Xia N, Yang Y, Peng DQ: Influence of vitamin D supplementation on plasma lipid profiles: a meta-analysis of randomized controlled trials. *Lipids Health Dis* 11:42, 2012
15. Andersen R, Brot C, Mejborn H, Molgaard C, Skovgaard LT, Trolle E, Ovesen L: Vitamin D supplementation does not affect serum lipids and lipoproteins in Pakistani immigrants. *Eur J Clin Nutr* 63:1150-1153, 2009
16. Wood AD, Secombes KR, Thies F, Aucott L, Black AJ, Mavroeidi A, Simpson WG, Fraser WD, Reid DM, Macdonald HM: Vitamin D3 supplementation has no effect on conventional cardiovascular risk factors: a parallel-group, double-blind, placebo-controlled RCT. *J Clin Endocrinol Metab* 97:3557-3568, 2012
17. Salehpour A, Shidfar F, Hosseinpanah F, Vafa M, Razaghi M, Hoshiarrad A, Gohari M: Vitamin D3 and the risk of CVD in overweight and obese women: a randomised controlled trial. *Br J Nutr* 108:1866-1873, 2012
18. Renzaho AM, Halliday JA, Nowson C: Vitamin D, obesity, and obesity-related chronic disease among ethnic minorities: a systematic review. *Nutrition* 27:868-879, 2011
19. Saneei P, Salehi-Abargouei A, Esmaillzadeh A: Serum 25-hydroxy vitamin D levels in relation to body mass index: a systematic review and meta-analysis. *Obes Rev* 14:393-404, 2013
20. Soares MJ, Chan SP-D, Ghanbari MH: Calcium and vitamin D for obesity: a review of randomized controlled trials. *Eur J Clin Nutr* 65:994-1004, 2011

21. von Hurst PR, Stonehouse W, Coad J: Vitamin D supplementation reduces insulin resistance in South Asian women living in New Zealand who are insulin resistant and vitamin D deficient - a randomised, placebo-controlled trial. *Br J Nutr* 103:549-555, 2010