Effect of vitamin D3 supplementation on insulin resistance and β-cell function in prediabetes: a double-blind, randomized, placebo-controlled trial


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Title:
Effect of vitamin D₃ supplementation on insulin resistance and β-cell function in prediabetes: a double-blind, randomised, placebo-controlled trial

Short title: Effect of vit D₃ supplementation on insulin action

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Abbreviations:
ADA American Diabetes Association
AUC Area under the curve
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>EGP</td>
<td>Endogenous Glucose Production</td>
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<tr>
<td>GIR</td>
<td>Glucose Infusion Rate</td>
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<tr>
<td>HOMA-B</td>
<td>Homeostasis model assessment of β-cell function</td>
</tr>
<tr>
<td>IFG</td>
<td>Impaired Fasting Glucose</td>
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<tr>
<td>IGT</td>
<td>Impaired Glucose Tolerance</td>
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<tr>
<td>IR</td>
<td>Insulin resistance</td>
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<tr>
<td>OGTT</td>
<td>Oral glucose tolerance test</td>
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<tr>
<td>Ra</td>
<td>Rate of appearance of glucose</td>
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<td>Rd</td>
<td>Rate of disappearance of glucose</td>
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Background

Observational studies have suggested an inverse association between low serum 25(OH)D concentrations and development of type 2 diabetes. High-quality trials are required to test the hypothesis that vitamin D is a direct contributor to type 2 diabetes pathogenesis.

Objective

The purpose of this double-blind randomised placebo-controlled trial was to investigate the effect of vitamin D₃ supplementation on insulin resistance (IR) and β-cell function in people with prediabetes and suboptimal vitamin D status (<50nmol/l).

Design

Sixty-six individuals were randomised to receive 3000IU (75µg) vitamin D₃ or placebo daily for 26 weeks. Compliance was monitored by pill count and change in serum 25(OH)D concentration using liquid chromatography-mass spectrometry. The primary endpoint was between-group difference in change in IR assessed using a two-step euglycaemic hyperinsulinaemic clamp combined with infusion of tritiated glucose. An oral glucose tolerance test was performed pre and post-intervention to calculate indices of β-cell function. Between group comparisons were made using analysis of covariance (ANCOVA).

Results

64 participants completed the study. Baseline serum 25(OH)D in the vitamin D₃ and placebo group was 30.7 and 30.0nmol/l with status increasing by 70.5nmol/l and 5.3nmol/l respectively (between group difference in vitamin D=65.8nmol/l (95% CI 54.2, 77.3, p<0.01), after supplementation. There was no difference between groups in measures of whole-body, peripheral or hepatic IR or in any measure of glycaemic control or β-cell function.

Conclusion
This study employed a robust assessment of IR and β-cell function and targeted a high-risk population with low 25(OH)D status at baseline and found that Vitamin D₃ supplementation had no effect on insulin action in people with prediabetes.

Keywords

Prediabetes, Insulin resistance, euglycaemic hyperinsulinaemic clamp, β-cell function, vitamin D
Introduction

Vitamin D is a fat soluble steroid molecule which binds to the vitamin D receptor in its active form. Expression of the vitamin D receptor in many diverse cell types (pancreatic islet cells, myocytes, hepatocytes and adipocytes) raises the suspicion that vitamin D may be involved in multiple cellular processes, including the body’s response to insulin. Insulin resistance (IR) is a key predictor of the development of type 2 diabetes and cardiovascular disease (CVD)(1,2) and interventions that reverse or prevent IR may help to attenuate the risk of these conditions.

Observational studies have demonstrated an inverse association between low serum 25(OH)D concentrations and an increased risk of developing type 2 diabetes(3-5). Such evidence has opened up the possibility that Vitamin D₃ supplementation could be a potentially straightforward and low-cost healthcare intervention to enhance glucose metabolism, attenuate IR and reduce the burden of type 2 diabetes. Initial randomised controlled trials (RCTs) exploring a potential cause-effect relationship between vitamin D and metabolic health had many major limitations. Results from inceptive systematic reviews of RCTs in this field(5-7) noted that many trials were either small or based on post hoc analyses of completed trials, that dosage and compliance may have been sub-optimal, and that participants started with a wide range of vitamin D levels at baseline. Thus, these systematic reviews concluded that high-quality RCTs conducted in well-defined populations specifically designed to test the hypothesis that vitamin D is a direct contributor to the pathogenesis, were needed(6,7). In terms of the well-defined population, insulin-resistant populations with sub-optimal vitamin D status are most likely to benefit from vitamin D supplementation. This was demonstrated by proof of concept findings from small RCTs showing an improvement in HOMA-IR and postprandial insulin sensitivity that used variable doses of vitamin D given for varying
Based on the evidence described above, a double-blind randomised placebo-controlled trial was designed to examine the effect of vitamin D₃ supplementation (3000IU daily) for 26 weeks on IR and β-cell function in people with prediabetes and suboptimal vitamin D status (<50nmol/l). Ethical approval for this trial was obtained from the Office for Research Ethics Committee Northern Ireland (ORECNI)(Project ID 117728) and the study protocol was registered on ClinicalTrials.gov (ID No. NCT01889810).

Subjects & Methods

Participant Screening and Recruitment

Participants were recruited from the general population using several advertising approaches including press releases, advertisements and recruitment from outpatient clinics. Interested participants were screened for eligibility between September 2013 and May 2015. The inclusion criteria were as follows: age >18 years, prediabetes as per American Diabetes Association (ADA) criteria (Impaired fasting glucose (IFG)(5.6-6.9mmol/l) and/or impaired glucose tolerance (IGT)(fasting plasma glucose <7.0 and 2-hour value 7.8-11.0mmol/l) diagnosed following oral glucose tolerance test (OGTT)), serum 25(OH)D <50nmol/l.

The exclusion criteria were as follows: diabetes mellitus, established CVD, surgery within the previous 3 months, psychiatric problems, medications or health problems known to affect vitamin D metabolism, the use of vitamin supplements, hypercalcaemia, renal calculi, renal impairment, hepatic impairment, excessive alcohol consumption, pregnancy or lactating.

Intervention
Prior to commencement of the study, a randomisation schedule was produced by an independent statistician, using a web-based tool (randomization.com). The randomisation was performed in blocks of six, to control for the effects of seasonal variation. The randomisation schedule was passed directly to a private pharmaceutical company (Victoria Pharmaceuticals, N. Ireland) who prepared the study medication in sealed white containers labelled with participant number. These bottles were then passed to the hospital pharmacy for dispensing and the researcher obtained the study medication for the corresponding participant from pharmacy, ensuring blinding of the researcher as to study group allocation. When a participant was recruited to the study and completed the consent form, they were allocated the next sequential study number.

Participants were randomly assigned to either the vitamin D$_3$ (3000IU cholecalciferol daily) or placebo group and were instructed to take one capsule daily for 26 weeks. A previous double-blind RCT of vitamin D$_3$ supplementation indicated that optimal 25(OH)D for reducing insulin resistance was 80-119 nmol/l(9) and Bischoff-Ferrari et al.(11) suggest that the most advantageous serum concentration of 25(OH)D for multiple health outcomes is between 90-100nmol/l and that supplementation at low doses, in the region of 400IU/day may not attain these targets in the majority of participants(11). A dose of 3000IU vitamin D$_3$ was chosen for the trial reported here based on the knowledge that 1000IU vitamin D$_3$ is required to bring vitamin D concentrations up to 75nmol/l in no less than 50% of the population. Based on the rule of thumb that each additional 100IU of vitamin D$_3$ per day raises serum 25(OH)D by approximately 2.5nmol/l, we calculated that a dose of 3000IU vitamin D$_3$ daily was required in order to attain optimal serum 25(OH)D concentration in a population with suboptimal vitamin D status(12).

Capsules of vitamin D$_3$ and placebo were provided in standard sealed white containers and were identical and tasteless to ensure both participants and investigators were blinded to the
group allocation. Supplementation at this dose has not been reported to be associated with adverse effects(13) and a daily dose has been shown to be more effective than weekly or monthly doses in terms of raising serum 25(OH)D concentrations(14,15). The researchers telephoned the participants on a six weekly basis to encourage compliance. Compliance was assessed by pill-count and change in serum 25(OH)D concentration.

Participants were advised to maintain their usual diet and exercise regimens.

Study Assessments

All assessments were performed at the Regional Centre for Endocrinology & Diabetes, Royal Victoria Hospital, Belfast.

The primary endpoint of the trial was group change in IR which was measured by HJW, who was unaware of the patient’s intervention group. A two-step euglycaemic-hyperinsulinaemic clamp combined with the infusion of [3-3\text{H}]glucose was carried out pre and post-intervention at weeks 0 and 26 as previously described(16). An adjusted primed continuous infusion of high-performance liquid chromatography purified [3-3\text{H}]glucose (based on fasting plasma glucose concentration) was administered during a 2-hour equilibration period. Insulin was infused at a constant rate during two sequential steps; 0.4mU/kg/min (Step 1, time 0-120min) followed by 2.0mU/kg/min (Step 2, time 120-240min). The right arm was placed in a temperature controlled plexiform heat box, maintained at 55°C to allow sampling of arterialised venous blood. Plasma glucose concentrations were measured at 5 minute intervals using a bedside glucose analyser and maintained at 5.8mmol/l by adjusting the rate of the exogenous 20% glucose infusion. Plasma for the measurement of glucose specific activity was deproteinized with barium hydroxide and zinc sulfate using the method of Somogyi(17). Aliquots of tracer infusate and labelled exogenous glucose infusion were spiked into nonradioactive plasma and processed in parallel to allow calculation of [3-3\text{H}]glucose
infusion rates (GIRs). IR was assessed using the exogenous GIR required to maintain
euglycaemia corrected for body weight. The isotope dilution method was used to allow the
measurement of endogenous glucose production (EGP), the rate of appearance of glucose in
the peripheral circulation (Ra) and the rate of disappearance or whole-body uptake of glucose
(Rd).

Secondary endpoints included measures of insulin sensitivity, β-cell function and the
disposition index derived from values obtained during a 75g OGTT performed pre and post-
intervention following an overnight fast. Insulin sensitivity was calculated using 1)HOMA-IR
and HOMA2-IR; 2)HOMA2%S; and 3)Matsuda index = 10,000/ √((Glu₀ x Ins₀)(Glu_mean x
Ins_mean)). Insulin secretion was assessed by six methods: 1)Homeostasis model assessment of
β-cell function (HOMA-B and HOMA2%B); 2)insulin area under the curve (AUC)/glucose
AUC (trapezoidal rule); 3)Stumvoll first phase insulin secretion=[1283+1.829(Ins₃₀)–
138.7(Glu₃₀)+3.772(Ins₀)]; 4)Stumvoll second phase insulin secretion = 287+0.4164(Ins₃₀)–
26.07(Glu₃₀)+0.9226(Ins₀); where Ins₃₀ and Glu₃₀ are insulin(pmol/l) and glucose(mmol/l) at
30 minutes of the OGTT and Ins₀ is fasting serum insulin; 5)the insulinogenic index
(IGI)=(Ins₃₀–Ins₀/Glu₃₀–Glu₀); and 6)the insulinogenic index adjusted for insulin
sensitivity=IGI/HOMA-IR. An oral disposition index was assessed by three methods;
1)DI=IGIxMatsuda index; 2)Insulin secretion sensitivity index-2 (ISSI-2) =Matsuda
Index(insulin AUC/glucose AUC); and 3)IGI x (1/ Ins₀).

Laboratory Analysis
Fasting blood samples were taken at week 0 and week 26. All samples were processed and
stored at -80°C within two hours of collection. Samples were paired (pre and post-
intervention) and analysed in batches to minimise variation between samples. Serum
25(OH)D concentration was measured by liquid chromatography-tandem mass spectrometry
(LC-MS/MS) with inter-assay and intra-assay CVs of <10%. Serum insulin, c-peptide and
PTH levels were measured using an electrochemiluminescence (ECLIA) immunoassay (Roche Diagnostics, West Sussex, UK). Plasma glucose concentrations were measured using a GM9 Glucose Analyzer (Analox) for clamp samples. Laboratory measurements of glucose were performed using an enzymatic colorimetric assay (Roche Diagnostics, West Sussex, UK). HOMA-IR was calculated using a minimum of three fasting samples. HOMA2-IR was calculated using the University of Oxford Clinical Trials Unit online calculator. Fasting venous blood samples were analysed for kidney and liver function tests, full blood counts and lipid profiles using commercial enzymatic immunoassays and processed by a quality assured laboratory. HbA1c was analysed using High Pressure Liquid Chromatography (HPLC). Commercial kits were used to analyse non-esterified fatty acids on the ILab-600 biochemical analyser.

Statistics
Statistical analyses were performed using IBM SPSS Statistics version 22 software. The distributions of variables were evaluated for normality by visual inspection of histograms. Data are presented as mean (standard deviation) pre and post-intervention for normally distributed variables. Categorical variables are presented as percentages. Pearsons correlation was performed to assess for correlation between serum 25(OH)D concentration, BMI and insulin resistance at baseline. ANCOVA was used to calculate the difference in mean outcome (and 95% CIs) between the intervention and placebo group adjusting for baseline levels as recommended(18). ANCOVA was also used to perform exploratory sub-group analyses for individuals who were most deficient at baseline (<25nmol/l) and reached the highest serum 25(OH)D concentrations (>80nmol/l) post-intervention to determine potential trends towards significance. A completers analysis was not carried out as only two individuals withdrew from the trial.
A pre-study sample size was conducted based upon a standard deviation of 9.3 mmol/kg/min for GIR(19), a total of 30 participants per group gave the study 80% power at the 5% level of significance to detect as statistically significant a difference of 6.8 mmol/kg/min (or approx. 17%) between groups. Factoring in a loss to follow-up of 10%, 66 participants were recruited with the aim of 60 completing the trial.

Results

In total, 308 participants attended a screening appointment between September 2013 and May 2015 to determine eligibility. Sixty-six participants were randomised (35 and 31 participants in the vitamin D₃ and placebo groups respectively) and 64 participants completed the 26-week protocol (Figure 1). There were two drop-outs between weeks 0 and 26; one from the vitamin D₃ group, because of personal reasons and one from the placebo group, because of illness unrelated to the study. No adverse events were associated with the intervention.

Baseline characteristics of both groups are presented in Table 1. The study population consisted of 59% males, with a mean age of 53.3 years. All participants were overweight or obese, (mean BMI 34.3 kg/m²), with an increased waist circumference (mean 113.1 cm) and elevated waist:hip ratio (mean 0.94)(20). Mean serum 25(OH)D concentration was similar in both the vitamin D₃ and placebo groups (30.7 nmol/l and 30.0 nmol/l respectively) at baseline. All patients had prediabetes defined as IFG and/or IGT on OGTT. Mean HbA₁c was 38.3 mmol/mol, which is approaching the ADA threshold of 38.8 mmol/mol for diagnosis of prediabetes. Recruitment to the study was approximately equal in both groups throughout winter and summer.

BMI and insulin resistance at baseline were directly correlated. There was no correlation between BMI and serum 25(OH)D concentration or serum 25(OH)D and insulin resistance at baseline.
Pill count suggested excellent compliance with the study medication (98.0% and 97.8% compliance in the vitamin D$_3$ and placebo groups respectively). Mean change in serum 25(OH)D concentration was significantly higher within the vitamin D$_3$ group compared to the change in serum 25(OH)D within the placebo group (70.5nmol/l versus 5.3nmol/l respectively; difference in mean between groups adjusted for baseline 65.8nmol/l (95% CI 54.2, 77.3; p<0.01)(Table 2). The goal of maintaining body weight was achieved; there was no statistically significant between group change in body weight, BMI, waist circumference or waist:hip ratio.

There was no statistically significant between groups in change in GIR, fasting plasma glucose, fasting serum insulin, HOMA-IR or HOMA2-IR following vitamin D$_3$ supplementation (Table 2). For instance, the mean step 1 GIR (corrected for absolute weight) increased by 0.9µmol/kg/min in the vitamin D$_3$ group and decreased by 0.4µmol/kg/min in the placebo group corresponding to a between group difference adjusting for baseline of 1.4µmol/kg/min (95%CI -0.6, 3.3; p=0.16). No statistically significant differences were observed in between group changes in EGP, Ra and Rd following the intervention (Table 3).

Sub-group analyses for individuals who were most deficient at baseline (<25nmol/l) and reached the highest serum 25(OH)D concentrations (>80nmol/l) post-intervention did not alter these results (data not shown).

Changes in glycaemic indices (fasting plasma glucose and serum insulin, 2 hour plasma glucose and insulin and HbA1c), insulin secretion (HOMA-B, HOMA2-%B, HOMA2-%S, Insulin AUC/glucose AUC, Stumvoll 1$^{st}$ and 2$^{nd}$ phase insulin secretion, the insulinogenic index and the insulinogenic index adjusted for insulin sensitivity), insulin sensitivity (HOMA-IR, HOMA2-IR and the Matsuda index) and the oral disposition index are presented.
in Table 4. Overall, there was no statistically significant difference between group change in any of these measures of glycaemia and β-cell function.

No statistically significant difference was observed between group change in non-esterified fatty acids (data not shown).

Discussion

This carefully designed double-blind randomised placebo-controlled trial tested the hypothesis that oral vitamin D₃ supplementation (3000IU daily) would reduce IR in a high-risk population with prediabetes and suboptimal vitamin D status within 26 weeks. The trial achieved high participant retention (97%) and a high level of compliance: a statistically significant increase in serum 25(OH)D concentration was observed in the vitamin D group and weight status remained stable throughout the study. There was, however, no effect of vitamin D₃ supplementation on euglycaemic clamp measures, glucose turnover assessed using the isotope dilution method, HOMA-IR, HOMA2-IR, fasting plasma glucose and serum insulin concentration, HbA1c and measures of β-cell function. Subgroup analyses of individuals with serum 25(OH)D concentrations <25nmol/l at baseline and in those attaining a 25(OH)D status >80nmol/l following the intervention did not alter the results. The results of this study provide convincing evidence that vitamin D₃ supplementation does not effect peripheral, whole-body or hepatic IR in people with prediabetes.

In the last few years, several other studies have reported no beneficial effect of vitamin D supplementation on measures of glycaemia and IR. However, many of these trials have at least one study design limitation that is not applicable to the trial presented here, including: the study of populations who were not vitamin D deficient(21-24) and the dosage of vitamin D supplements was not sufficient to attain optimal vitamin D status(25,26).
The failure to focus on participants with suboptimal vitamin D status is commonly cited as a reason to explain the lack of benefit of vitamin D supplementation. One RCT reported significant improvement in insulin sensitivity and IR in 81 South Asian females who were given 4000IU vitamin D₃ daily for six months. The mean serum 25(OH)D was 21nmol/l at baseline(9). The results from this study would support the hypothesis that participants who are vitamin D deficient are most likely to derive benefit and demonstrate a response following supplementation. However, there are only a few trials in which the inclusion criteria have stipulated participants should be vitamin D deficient at baseline. In a systematic review, participants in only one of the fifteen RCTs analysed had a mean baseline serum 25(OH)D less than 30nmol/l(27). More recent trials have stipulated the inclusion of participants with suboptimal vitamin D status, but in many cases the threshold for inclusion is a serum 25(OH)D concentration <75nmol/l, which still may not be low enough to demonstrate a beneficial response to supplementation. In addition, a number of the trials which have failed to demonstrate a beneficial response to vitamin D supplementation have performed a secondary analysis to include those who are vitamin D deficient at baseline(23,24,28). However, the secondary analysis tends to include small numbers and thus has limited power. As part of the inclusion criteria for this study, participants had to have suboptimal status defined as 25(OH)D <50nmol/l and the mean serum 25(OH)D concentration at baseline was 30.3nmol/l. We also performed a secondary analysis for participants who were vitamin D deficient (<25nmol/l) at baseline and noted no improvement in insulin resistance or β-cell function following vitamin D supplementation. To our knowledge, four RCTs have also stipulated that eligible participants should have serum 25(OH)D concentrations <50nmol/l at baseline (mean serum 25(OH)D concentration was 25.0nmol/l, 31.4nmol/l, 33.3nmol/l and 38.0nmol/l) and they also report no effect of vitamin D₃ supplementation on IR when assessed using OGTT and the euglycaemic-
hyperinsulinaemic clamp(26,29-31). These studies, however, were of shorter duration (8 weeks(30) and 16 weeks(26,29)) and mean serum 25(OH)D concentration post-intervention reached only 47nmol/l(30) and 60nmol/l(26), which falls short of the proposed optimal concentration in the range of 80-119nmol/l for non-skeletal health(11).

The vitamin D group in this study demonstrated a high compliance with the study protocol attaining a mean serum 25(OH)D 101.3nmol/l post-intervention. The majority of participants in the vitamin D₃ group attained serum 25(OH)D levels greater than 80nmol/l (27/34 participants) and almost half of the participants in the vitamin D₃ group (16/34 participants) had serum 25(OH)D greater than 100nmol/l at the end of the intervention period. A secondary analysis on those participants who attained a serum 25(OH)D concentration greater than 80nmol/l was performed and failed to demonstrate any beneficial response in measures of IR and β-cell function. These results are consistent with other recent studies that attained mean serum 25(OH)D concentrations in excess of 100nmol/l post-intervention, using vitamin D₃ supplementation (20,000-88,865IU weekly), but found no effect on IR assessed using HOMA-IR and the insulin sensitivity index or incident diabetes(23,32). The maximum serum 25(OH)D concentration noted in our study was 161.6nmol/l and serum corrected calcium remained within the normal range during the intervention period with no adverse events reported. This suggests that vitamin D₃ supplementation at the dose used in this study was safe. Bischoff-Ferrari et al. suggest that toxicity occurs with serum 25(OH)D levels in excess of 240nmol/l, which may be reached if intakes exceed 100,000IU per day(33).

The majority of intervention studies investigating the effect of vitamin D₃ supplementation on IR have done so using mathematical indices such as HOMA-IR and QUICKI, which are calculated using fasting plasma glucose and serum insulin concentrations. These indices may be affected by errors in measurements, particularly if a single fasting sample is used. In this study, we calculated HOMA-IR values using the average value for plasma glucose and serum
insulin of a minimum of three samples. To our knowledge, this is the only RCT to investigate the effect of high dose vitamin D\textsubscript{3} supplementation on IR in a high-risk cohort with prediabetes and suboptimal levels of vitamin D using the two-step euglycaemic-hyperinsulinaemic clamp technique. Four other RCTs have used the euglycaemic-hyperinsulinaemic clamp to investigate the effect of vitamin D\textsubscript{3} supplementation and reported no change in IR. Two of these studies have been limited by duration (7 days\textsuperscript{34} to 8 weeks\textsuperscript{30}), small sample sizes (12\textsuperscript{30} to 18\textsuperscript{34} participants) and the inclusion of young, healthy individuals with normal glucose tolerance or type 2 diabetes\textsuperscript{30,31,34}. The fourth RCT to use the euglycaemic-hyperinsulinaemic clamp reported similar findings to our group and demonstrated no effect of vitamin D\textsubscript{3} supplementation (100000IU bolus dose, followed by 4000IU daily for 16 weeks, serum 25(OH)D concentration post-intervention 88.4+/−21.0nmol/l) on IR or insulin secretion in 54 vitamin D deficient (<50nmol/l), overweight or obese adults\textsuperscript{29}. The study protocol details that participants were not diabetic, but there are no further details regarding glucose tolerance. Our protocol employed a two-step euglycaemic-hyperinsulinaemic clamp with infusion of tritiated glucose thus enabling a more comprehensive assessment of IR, including hepatic, peripheral and whole-body insulin resistance than the study by Mousa et al. which employed a one-step clamp without infusion of a tracer.

The duration of this study (26 weeks) was chosen to allow sufficient time for optimisation of vitamin D status and because the primary endpoint is known to respond quickly (within 2-6 weeks) to dietary intervention\textsuperscript{35-38}. The longest running reported RCT to date investigated the effect of vitamin D supplementation in 511 participants with prediabetes for five years. This study reported no change in glucose levels, IR using HOMA-IR, progression to type 2 diabetes, serum lipids and blood pressure after supplementation with 20,000IU vitamin D\textsubscript{3} on a weekly basis for five years compared to placebo\textsuperscript{23}. 
Study strengths and limitations

This was a well powered double-blind randomised placebo-controlled trial designed to examine a specific question using robust measures of assessment of IR and serum 25(OH)D.

The euglycaemic-hyperinsulinaemic clamp studies were performed to a high standard by a single investigator (HJW) and an experienced clamp technician (CNE) using an established protocol(16). Pre and post-intervention serum and plasma samples were stored at -80°C and analysed in pairs to minimise analytic inter-assay variability.

Based on the needs identified in previous studies and expert systematic reviews, we studied a high-risk cohort of participants as they were the most likely group of individuals to demonstrate a response to supplementation. Eligible participants were identified using strict inclusion/exclusion criteria based on standard clinical guidelines published by the ADA and Institute of Medicine and the recruitment target was met. Participants were recruited from a wide variety of avenues and included males and females from across a range of ages.

Participant retention and compliance was very high and there were no safety concerns noted at any point during the intervention. Recruitment was ongoing over a 22 month period and equal numbers of participants were recruited throughout the seasons and randomised in blocks, controlling for any effect of seasonal variation. There was no change in weight during the study thus minimising the effects of this possible confounder.

The main study limitation is that participants were predominantly Caucasian (98%) and were recruited in a single centre, raising the possibility that results may not be applicable in other more diverse populations. This is unlikely however, as the results we present are consistent with the findings of recently published RCTs performed elsewhere.

Future work
This study recruited participants with a serum 25(OH)D concentrations <50nmol/l at baseline, as it was proposed that this was the group of individuals who would benefit most from vitamin D supplementation. The Scientific Advisory Committee on Nutrition (SACN) have recommended that future studies should investigate the effect of vitamin D supplementation in populations with lower serum 25(OH)D concentrations (20-30nmol/l)(39). We performed subgroup analysis of those with the lowest basal, and greatest increase in, serum 25(OH)D concentration and did not observe any trend towards significance for measures of glycaemia, IR or β-cell function. Thus it appears, from this and recent trials(26,29,30), unlikely that further studies of participants with lower serum 25(OH)D concentrations will demonstrate a positive effect following supplementation.

Recent trial sequential meta-analyses and very large Mendelian randomisation studies report no effect of vitamin D supplementation on skeletal, vascular or cancer outcomes and the development of type 2 diabetes(40-42). It is possible that any beneficial effect that vitamin D may have on diabetes risk may be mediated by mechanisms other than IR. Results from four large ongoing multicentre trials (D2d (Vitamin D and Type 2 Diabetes), FIND (Finnish Vitamin D Trial), VITAL (Vitamin D and Omega-3 Trial) and D-HEALTH)(43-46) with sample size ranging from 2,382 to 28,875 individuals, investigating the effect of vitamin D₃ supplementation (1600IU to 4000IU daily or the equivalent weekly or monthly dose) will provide further and perhaps conclusive evidence to answer the question of whether vitamin D on its own, or along with omega-3 fatty acids, can reduce the incidence of type 2 diabetes.

Implications

In light of current evidence, universal screening for vitamin D deficiency in people at risk of type 2 diabetes is not warranted and should be reserved for individuals who are generally at high risk of deficiency (including malabsorptive states, post-bariatric surgery, currently
prescribed medication known to affect vitamin D metabolism and individuals with limited
sun exposure) or individuals with metabolic bone disease.

Conclusions

There is no doubt that vitamin D is important for the maintenance of skeletal health and there
has been much interest in studies suggesting that vitamin D may be beneficial for a number of
other health outcomes, including the prevention of type 2 diabetes.

This robust double-blind, randomised, placebo-controlled trial found that optimising vitamin
D status in people with prediabetes and suboptimal vitamin D concentrations did not have an
effect on IR and β-cell function. In light of these results, and those described in recently
published RCTs, it is unlikely that vitamin D₃ supplementation has a direct role in the
pathogenesis of type 2 diabetes.
Author Contributions

HJW carried out all assessments of IR, assisted with analysis of clamp samples, performed statistical analysis, and drafted the manuscript.

LH assisted with study recruitment, managing day-to-day running of study and analysis of serum 25(OH)D samples, reviewed and approved the final version of the manuscript.

CNE assisted with all assessments of IR, performed analysis of clamp samples, reviewed and approved the final version of the manuscript.

CC was the study statistician and supervised procedures for randomisation and blinding, supervised statistical analysis, reviewed and approved the final version of the manuscript.

JVW contributed to the design of the work, revising the manuscript for important intellectual content and approving the final version.

ISY contributed to the design of the work, revising the manuscript for important intellectual content and approving the final version.

PMB contributed to the design of the work, revising the manuscript for important intellectual content and approving the final version.

SJH contributed to the conception and design of the study, oversaw the assessment of the primary endpoint, contributed to the results and conclusions and reviewed and edited the final version of the manuscript.

MCM, the principal investigator, contributed to the conception and design of the study, oversaw the assessment of the study endpoints, contributed to all parts of manuscript, and reviewed and edited the manuscript. MCM acts as guarantor of this work.
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Parts of this study were presented in abstract form at the Irish Endocrine Society Annual Scientific Meeting, Belfast 19\textsuperscript{th}-20\textsuperscript{th} October 2016, Diabetes UK Annual Professional Conference 8-10\textsuperscript{th} March 2017 and the 77th Scientific Sessions of the American Diabetes Association, San Diego, 9-13 June 2017. The authors thank all the participants in the study for their time, interest, cooperation, and contribution to this research.


(22) Moreira-Lucas TS, Duncan AM, Rabasa-Lhoret R, Vieth R, Gibbs AL, Badawi A, Wolever TMS. Effect of vitamin D supplementation on oral glucose tolerance in individuals with low vitamin D status and increased risk for developing type 2 diabetes (EVIDENCE): A double-blind, randomized, placebo-controlled clinical trial. Diabetes, Obesity and Metabolism 2016:n/a-n/a.


(42) Wang N, Zhao L, Chen C, Chen Y, Lu Y. Vitamin D, Prediabetes, and Diabetes—Bidirectional Mendelian Randomization Analysis. Diabetes 2018(67 (Supplement 1)).


<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall n=66</th>
<th>Vitamin D₃ n=35</th>
<th>Placebo n=31</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male, % (n)</td>
<td>59.1 (39)</td>
<td>54.3 (19)</td>
<td>64.5 (20)</td>
</tr>
<tr>
<td>Age, y</td>
<td>53.3 (10.6)</td>
<td>52.4 (2.0)</td>
<td>54.0 (1.7)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>34.3 (6.9)</td>
<td>34.7 (8.0)</td>
<td>33.9 (5.6)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>113.1 (10.8)</td>
<td>111.6 (11.3)</td>
<td>114.5 (10.2)</td>
</tr>
<tr>
<td>Waist to hip ratio</td>
<td>0.94 (0.07)</td>
<td>0.93 (0.06)</td>
<td>0.96 (0.11)</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>134 (14)</td>
<td>133 (11)</td>
<td>135 (16)</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>82 (8)</td>
<td>82 (8)</td>
<td>82 (7)</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>5.0 (1.1)</td>
<td>5.0 (1.1)</td>
<td>5.1 (1.1)</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/l</td>
<td>1.3 (0.3)</td>
<td>3.8 (1.3)</td>
<td>4.2 (1.5)</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/l</td>
<td>3.0 (0.9)</td>
<td>2.9 (0.8)</td>
<td>3.0 (1.0)</td>
</tr>
<tr>
<td>Current smoker, % (n)</td>
<td>7.6 (5)</td>
<td>11.4 (4)</td>
<td>3.2 (1)</td>
</tr>
<tr>
<td>Ex-Smoker, % (n)</td>
<td>34.4 (21)</td>
<td>31.4 (11)</td>
<td>32.3 (10)</td>
</tr>
<tr>
<td>Fasting plasma glucose, mmol/l</td>
<td>5.6 (0.7)</td>
<td>5.5 (0.6)</td>
<td>5.7 (0.7)</td>
</tr>
<tr>
<td>2 hr plasma glucose, mmol/l</td>
<td>7.7 (1.9)</td>
<td>7.4 (1.9)</td>
<td>7.8 (1.9)</td>
</tr>
<tr>
<td>HbA1c, mmol/mol</td>
<td>38.3 (4.6)</td>
<td>37.4 (4.2)</td>
<td>39.3 (5.0)</td>
</tr>
<tr>
<td>FHx Diabetes, % (n)</td>
<td>43.9 (29)</td>
<td>48.6 (17)</td>
<td>38.7 (12)</td>
</tr>
<tr>
<td>IFG, % (n)</td>
<td>45.5 (30)</td>
<td>48.6 (17)</td>
<td>41.9 (13)</td>
</tr>
<tr>
<td>IGT, % (n)</td>
<td>31.8 (21)</td>
<td>31.4 (11)</td>
<td>32.3 (10)</td>
</tr>
<tr>
<td>IFG&amp;IGT, % (n)</td>
<td>22.7 (15)</td>
<td>20 (7)</td>
<td>25.8 (8)</td>
</tr>
<tr>
<td>Serum 25(OH)D, nmol/l</td>
<td>30.3 (14.7)</td>
<td>30.7 (14.3)</td>
<td>30.0 (15.4)</td>
</tr>
<tr>
<td>Season of entry-Winter % (n)</td>
<td>53 (35)</td>
<td>54.3 (19)</td>
<td>51.6 (16)</td>
</tr>
<tr>
<td>Season of entry-Summer % (n)</td>
<td>47 (31)</td>
<td>45.7 (16)</td>
<td>48.4 (15)</td>
</tr>
</tbody>
</table>

¹Variables are summarised as mean (standard deviation) or % (n)

Abbreviations: BMI = Body Mass Index; BP = Blood Pressure; FHx = Family history; HbA1c = glycated haemoglobin; HDL = High Density Lipoprotein; IFG = Impaired fasting glucose; IGT = Impaired glucose tolerance; LDL = Low Density Lipoprotein; 25(OH)D = 25-hydroxyvitamin D
Table 2: Insulin resistance at week 0 (pre-intervention) and week 26 (post-intervention) according to study group allocation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vitamin D3 (n=35)</th>
<th>Placebo (n=31)</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 26</td>
<td>Within group change</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.7 (8.0)</td>
<td>35.2 (8.5)</td>
<td>0.4</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/l)</td>
<td>30.7 (14.3)</td>
<td>101.3 (27.4)</td>
<td>70.5</td>
</tr>
<tr>
<td>Serum PTH (pg/ml)</td>
<td>49.8 (18.9)</td>
<td>42.1 (14.2)</td>
<td>-8.0</td>
</tr>
<tr>
<td>Fasting plasma glucose concentration (mmol/l)</td>
<td>6.0 (0.5)</td>
<td>6.0 (0.5)</td>
<td>0.0</td>
</tr>
<tr>
<td>Fasting serum insulin concentration (mU/l)</td>
<td>14.8 (9.7)</td>
<td>16.3 (10.3)</td>
<td>1.2</td>
</tr>
<tr>
<td>Step 1 serum insulin concentration (mU/l)</td>
<td>51.3 (12.5)</td>
<td>52.7 (12.8)</td>
<td>1.5</td>
</tr>
<tr>
<td>Step 2 serum insulin concentration (mU/l)</td>
<td>253.2 (56.6)</td>
<td>253.6 (53.0)</td>
<td>0.4</td>
</tr>
<tr>
<td>Step 1 GIR corrected for absolute weight (µmol/kg/min)</td>
<td>9.4 (4.3)</td>
<td>10.2 (5.7)</td>
<td>0.9</td>
</tr>
<tr>
<td>Step 2 GIR corrected for absolute weight (µmol/kg/min)</td>
<td>37.1 (12.3)</td>
<td>37.4 (13.8)</td>
<td>0.3</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>37.4 (4.2)</td>
<td>39.3 (4.5)</td>
<td>1.8</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>4.0 (3.0)</td>
<td>4.4 (3.0)</td>
<td>0.3</td>
</tr>
<tr>
<td>HOMA2-IR</td>
<td>2.0 (1.2)</td>
<td>2.2 (1.3)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Data are mean (SD), except for change, which is mean of difference between week 0 and week 26 values. ANCOVA presented as difference in mean between groups at week 26 (95% CI) adjusted for baseline.

Abbreviations: BMI = Body Mass Index; GIR = Glucose Infusion Rate; HOMA-IR = Homeostatic Assessment of Insulin Resistance; PTH = Parathyroid Hormone
Table 3: Glucose production and glucose uptake at week 0 (pre-intervention) and week 26 (post-intervention) according to study group allocation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vitamin D3 (n=35)</th>
<th>Placebo (n=31)</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 26</td>
<td>Within group change</td>
</tr>
<tr>
<td>Ra Basal (µmol/kg/min)</td>
<td>9.5 (2.8)</td>
<td>8.6 (1.7)</td>
<td>-0.9</td>
</tr>
<tr>
<td>Ra Step 1 (µmol/kg/min)</td>
<td>13.8 (5.3)</td>
<td>14.0 (6.2)</td>
<td>0.2</td>
</tr>
<tr>
<td>Ra Step 2 (µmol/kg/min)</td>
<td>42.8 (13.8)</td>
<td>40.4 (13.6)</td>
<td>-2.5</td>
</tr>
<tr>
<td>EGP Basal (µmol/kg/min)</td>
<td>9.5 (2.8)</td>
<td>8.6 (1.7)</td>
<td>-0.9</td>
</tr>
<tr>
<td>EGP Step 1 (µmol/kg/min)</td>
<td>4.4 (2.1)</td>
<td>3.8 (1.9)</td>
<td>-0.6</td>
</tr>
<tr>
<td>EGP Step 2 (µmol/kg/min)</td>
<td>6.0 (3.2)</td>
<td>3.8 (3.9)</td>
<td>-2.3</td>
</tr>
<tr>
<td>Rd Basal (µmol/kg/min)</td>
<td>9.7 (2.5)</td>
<td>9.0 (1.6)</td>
<td>-0.7</td>
</tr>
<tr>
<td>Rd Step 1 (µmol/kg/min)</td>
<td>14.3 (5.1)</td>
<td>14.2 (5.9)</td>
<td>-0.1</td>
</tr>
<tr>
<td>Rd Step 2 (µmol/kg/min)</td>
<td>42.6 (13.7)</td>
<td>40.2 (13.7)</td>
<td>-2.4</td>
</tr>
</tbody>
</table>

Data are mean (SD), except for change, which is mean of difference between week 0 and week 26 values. ANCOVA presented as difference in mean between groups at week 26 (95% CI) adjusted for baseline.

Abbreviations: EGP = Endogenous Glucose Production; Ra = Rate of appearance of glucose; Rd = Rate of disappearance of glucose
Table 4: Measures of β-cell function at week 0 (pre-intervention) and week 26 (post-intervention) according to study group allocation

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vitamin D3 (n=35)</th>
<th>Placebo (n=31)</th>
<th>ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 0</td>
<td>Week 26</td>
<td>Within group change</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>5.6 (0.6)</td>
<td>5.7 (0.6)</td>
<td>0.1</td>
</tr>
<tr>
<td>2hr glucose (mmol/l)</td>
<td>7.4 (1.9)</td>
<td>7.4 (2.1)</td>
<td>0.0</td>
</tr>
<tr>
<td>Fasting serum insulin (mU/l)</td>
<td>17.2 (9.2)</td>
<td>18.9 (11.2)</td>
<td>1.5</td>
</tr>
<tr>
<td>2hr insulin (mU/l)</td>
<td>114.2 (113.0)</td>
<td>113.2 (99.2)</td>
<td>3.4</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>37.4 (4.2)</td>
<td>39.3 (4.5)</td>
<td>1.8</td>
</tr>
<tr>
<td>HOMA-B</td>
<td>174.3 (88.3)</td>
<td>176.5 (88.9)</td>
<td>1.5</td>
</tr>
<tr>
<td>HOMA2-%B</td>
<td>132.0 (44.0)</td>
<td>132.9 (45.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>HOMA2-%S</td>
<td>55.2 (24.4)</td>
<td>52.9 (26.4)</td>
<td>-1.4</td>
</tr>
<tr>
<td>Insulin AUC/glucose AUC</td>
<td>11.6 (5.6)</td>
<td>12.2 (5.4)</td>
<td>1.2</td>
</tr>
<tr>
<td>Stumvoll 1st phase</td>
<td>1619.7 (695.5)</td>
<td>1615.1 (785.0)</td>
<td>-14.0</td>
</tr>
<tr>
<td>Stumvoll 2nd phase</td>
<td>421.0 (159.8)</td>
<td>423.0 (182.0)</td>
<td>-0.6</td>
</tr>
<tr>
<td>Insulinogenic Index (IGI)</td>
<td>1.4 (1.3)</td>
<td>1.3 (1.2)</td>
<td>-0.1</td>
</tr>
<tr>
<td>Insulinogenic Index adjusted for insulin sensitivity</td>
<td>0.9 (0.9)</td>
<td>0.4</td>
<td>-0.5</td>
</tr>
<tr>
<td>Matsuda Index</td>
<td>2.5 (1.2)</td>
<td>2.5 (1.4)</td>
<td>-0.1</td>
</tr>
<tr>
<td>Disposition Index</td>
<td>3.7 (4.3)</td>
<td>3.3 (4.7)</td>
<td>-0.1</td>
</tr>
<tr>
<td>ISSI-2</td>
<td>24.7 (9.1)</td>
<td>26.1 (12.1)</td>
<td>2.3</td>
</tr>
<tr>
<td>IG1 x 1/FSI</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Data are mean (SD), except for change, which is mean of difference between week 0 and week 26 values. ANCOVA presented as difference in mean between groups at week 26 (95% CI) adjusted for baseline. Abbreviations: HOMA2-%B = HOMA of β-cell function; HOMA2-%S = HOMA of insulin sensitivity; ISSI-2 = Insulin Secretion Sensitivity Index-2
Figure Legend

Figure 1: Summary of flow of participants through the study