A Randomized Controlled Trial of High-Dose Vitamin D2 Followed by Intranasal Insulin in Alzheimer’s Disease

Mark S. Stein\textsuperscript{a,b,*}, Samuel C. Scherer\textsuperscript{d,e}, Kylie S. Ladd\textsuperscript{d} and Leonard C. Harrison\textsuperscript{a,b,c}

\textsuperscript{a}Royal Melbourne Hospital, Parkville, VIC, Australia
\textsuperscript{b}Walter and Eliza Hall Institute of Medical Research, Parkville, VIC, Australia
\textsuperscript{c}University of Melbourne, Parkville, VIC, Australia
\textsuperscript{d}Royal Freemasons Homes of Victoria, Melbourne, VIC, Australia
\textsuperscript{e}Cognitive, Dementia and Memory Service, Eastern Health Melbourne, Box Hill, VIC, Australia

Abstract. Poor vitamin D nutrition is linked with dementia, but vitamin D has not been tested in a randomized controlled trial (RCT) in Alzheimer’s disease (AD). Nasal insulin acutely improves cognition and vitamin D upregulates insulin receptor expression and enhances insulin action. In an RCT we examined the effect of high-dose vitamin D followed by nasal insulin on memory and disability in mild-moderate AD. 63 community-dwelling individuals aged >60 were recruited; 32 with mild-moderate disease (Folstein Mini-Mental State Examination [MMSE] score 12–24) met entry criteria and were randomized. All took low-dose vitamin D (1000IU/day) throughout. After run-in (8 weeks), they were randomized to additional high-dose D/placebo for 8 weeks, followed immediately by randomization to nasal insulin (60 IU qid)/placebo for 48 h. Primary outcome measures were Alzheimer’s disease assessment scale-cognitive subscale (ADAS-cog) and Disability Assessment in Dementia (after high-dose D) and ADAS-cog and Wechsler Memory Scale-Revised Logical memory (WMS-R LM) for immediate and delayed recall (after nasal insulin). Baseline median (interquartile range, IR) age, MMSE, and ADAS-cog were 77.5 (69–80), 19.5 (17–22), and 25.5 (20–31), respectively. Median 25OHD increased from 49 to 60 nM (\(p<0.01\)) after run-in and was 187 nM after high-dose vitamin D and 72 nM after placebo (\(p<0.001\)). Neither cognition nor disability changed significantly after high-dose D. ADAS-cog improved by a median (IR) of 9 (1–11) with nasal insulin after placebo high-dose vitamin D (\(p=0.02\)), but may represent regression to the mean as WMS-R LM did not change. We conclude that high-dose vitamin D provides no benefit for cognition or disability over low-dose vitamin D in mild-moderate AD.

Keywords: Alzheimer’s disease, nasal insulin, randomized controlled trial, vitamin D

INTRODUCTION

Dementia has been associated with poor vitamin D nutrition, and serum 25-hydroxyvitamin D (25OHD) concentration is reduced in dementia and correlates with impaired cognitive function [1–5]. However, randomized controlled trials (RCTs) of vitamin D on cognition or memory have not been reported. Cerebrospinal fluid (CSF) insulin is reduced in moderate-severe Alzheimer’s disease (AD), despite a higher concentration in plasma [6]. Intravenous insulin increases CSF insulin and acutely improves memory [7, 8] but is impractical for routine use. On the other hand, nasal insulin, which enters the CSF [9] but is impractical for routine use. On the other hand, nasal insulin, which enters the CSF [9] but is impractical for routine use. On the other hand, nasal insulin, which enters the CSF [9] but is impractical for routine use. On the other hand, nasal insulin, which enter CSF [9] but does not systemic circulation without absorption promoting agents [10], improves word recall and mood in healthy volunteers [11–13] and declarative memory in AD [14, 15], but the studies of nasal insulin in AD have
not been blinded. Because vitamin D may increase insulin receptor expression and reduce insulin resistance [16–18], we hypothesized that vitamin D and nasal insulin might synergize to benefit cognition and memory in AD. We performed an RCT to test, first, if adding high-dose vitamin D2 to ongoing low-dose vitamin D2 supplementation would improve cognition and disability in mild-moderate AD and, second, if nasal insulin immediately after high-dose vitamin D2 would further improve memory.

MATERIALS AND METHODS

Feasibility pilot study

Community dwelling participants age ≥60 were conversant in English, had mild-moderate AD (Folstein Mini-Mental State Examination [MMSE] [19] score 12–24) and, if taking an anti-cholinesterase agent were on a maintenance dose >3 months or, having failed therapy, were off agent >3 months. Exclusions were coexistent cerebrovascular disease, dysphasia, abnormalities on blood screening, prior cranial surgery, epilepsy, multiple sclerosis, type 1 diabetes, renal calculi, malabsorption; poorly-controlled psychiatric disorder, alcohol intake considered excessive or any other condition that could impair compliance or cognitive assessment.

Written informed consent was obtained from participants and next of kin/carer, and the Victorian Civil and Administrative Tribunal, and the study was approved by Melbourne Health Human Research Ethics Committee.

Screening tests were performed on fasting venous blood. Plasma calcium, albumin, uric acid, and creatinine were measured on the Olympus 2700 (Olympus, Tokyo). Corrected calcium (mM) was calculated as total calcium (mM) + (40-albumin [g/l] × 0.02). Individuals with hypercalcemia (corrected calcium > 2.60 mM) or creatinine > 0.2 mM or hyperuricemia (uric acid above the gender-matched reference range) were excluded and referred to family physicians.

The serum 25OHD radioimmunoassay (Diasorin, Stillwater, MN) measures 25OHD2 and 25OHD3 [20] with coefficients of variation (CVs) at median 32, 60, and 117 nM of 9.5, 9.5 and 9.0%. PTH was measured by Immulite 2000 Intact (Siemens Los Angeles, CA) with CVs at median 3.4, 30, and 98 pM of 8, 8 and 7.5%. Individuals with 25OHD > 90 nM were excluded to preserve study sensitivity [21].

For baseline assessment, a neuropsychologist recorded the Disability Assessment in Dementia Questionnaire (DAD) based on next of kin responses [23]. Higher ADAS-cog and DAD indicate, respectively, worse cognition and less disability. There were no other assessments of subjective improvement in relatives or participants.

Treatment was open label 3000 IU vitamin D2 tablets (Cardinal Health, Braeside, Victoria) for 8 weeks, with dose adjustments to maintain 25OHD 135–160 nM.

Fasting venous blood was monitored for 25OHD at 1, 2, 3, 4, 6, and 8 weeks, calcium and albumin at 4 and 8 weeks, and PTH, uric acid and creatinine at 8 weeks. After 8 weeks, ADAS-cog and DAD were repeated.

Randomized controlled trial

Inclusion and exclusion criteria were as for the pilot study, except that cerebrovascular disease was not excluded in order to broaden the clinical relevance of the RCT. Consent and ethics approval were given as for the pilot study. Blood screening was also as per the pilot study.

An occupational therapist (OT) recorded MMSE. A neuropsychologist applied ADAS-cog and Wechsler Memory Scale-Revised Logical memory (WMS-R LM) subtest for immediate and 30 minute delayed recall of two 25 information bit stories [24] and The Geriatric Depression Scale (GDS) [25]. The OT recorded, age, gender, medications, demographic, and anthropometric data, the DAD questionnaire reported by a next of kin and the Brief Pain Inventory (BPI) [26] using a verbal modification that enhances completion rate, reliability, and validity [27]. The same neuropsychologist and OT performed subsequent assessments.

Participants received one capsule daily of 1000IU vitamin D2 (‘Ostelin’, Boots, North Ryde, NSW) during an 8-week low-dose vitamin D run-in, following which ADAS-cog, WMS-R LM, GDS, and DAD were repeated, and venepuncture performed for the same analytes as at screening.

An off-site statistician then computer randomized to high-dose D/placebo capsules (6000 IU vitamin D2, Cardinal Health, Braeside, Victoria) blocking on AD treatment (donepezil, galantamine, none, other) (Fig. 1). Initial dosing was 2 capsules 3 times daily with food, and subsequently 0 to 2 capsules 3 times daily, reflecting empiric adjustment based on serum 25OHD at 2, 4, and 6 weeks, aiming to maintain 25OHD 130–175 nM. In a separate random allocation, each participant on high-dose vitamin D was paired with a ‘buddy’ on placebo. With every change in high-
dose vitamin D dose the ‘buddy’ was contacted to make the same capsule dose change. One investigator was unblinded to advise the pharmacist of dose adjustments. The pharmacist remained blinded and contacted participants.

All participants continued daily open-label low-dose (1000 IU) vitamin D2 as it was considered unethical for some participants to be on placebo alone given other potential benefits of D. Thus, the randomized phase tested for benefit from additional high-dose D. D2 was used because, apart from its extra-skeletal efficacy [21], it has an additional pathway through 24OHD [28] and its plant origin minimizes risk of transmissible disease. Base D2, rather than the calcemic metabolite, calcitriol, permits physiologic metabolism of circulating 25OHD to a range of active metabolites [29].

Primary endpoints were ADAS-cog, WMS-R LM immediate and delayed scores, GDS, and DAD. Secondary endpoints were ADAS-cog word recognition and word recall sub-scores, and DAD sub-scores of activities of daily living.

Immediately following the randomized high-dose D/placebo phase (Fig. 1), half of each high-dose vitamin D or placebo group received nasal insulin and half nasal placebo. Randomization was stratified by MMSE (<22 versus ≥22) then by drug treatment (none versus some). This allowed testing for the interaction of preceding high-dose vitamin D with nasal insulin.

Human insulin was Humulin-R (100 IU per ml, Eli Lilly, Indianapolis, IN). Placebo was Humulin-R diluent, prepared by Pharmalab (Lane Cove, NSW). Ten ml syringes were sterile filled with insulin or placebo by Pharmatel Fresenius Kabi (West Melbourne, Victoria). Nasal spray pump caps (code 73673; Pfeiffer, Germany) and 10 ml brown glass bottles (code 69546; Pfeiffer, Germany) were packed by Annex (Mulgrave, Victoria), gamma irradiated (Steritech, Dandenong, Victoria), and checked for sterility by ConsuChem (Dandenong, Victoria).

Before each treatment, the syringe content was transferred into a single-use bottle and a disposable pump cap attached. The pump was primed until a uniform volume (100 μl) of spray was released. Three sprays per nostril (total 600 IU insulin) were administered four times daily. The same pharmacist instructed administration and supervised the first dose. Next of kin/carers were given syringes, bottles and caps, and instructions for storage and use.

Fig. 1. Trial design and flow chart.
WMS-R LM story was told approximately one hour before the first nasal treatment and delayed recall recorded at approximately one hour and at one hour forty minutes after insulin. The story was repeated and delayed recall tested after a further 30 min and 48 h.

The WMS-R LM story was repeated and delayed recall tested at approximately one hour and at one hour forty minutes after insulin. The story was repeated and delayed recall tested after a further 30 min and 48 h. The story was then repeated and immediate and 30 min delayed recall recorded together with ADAS-cog, GDS and BPI.

Primary endpoints were ADAS-cog and WMS-R LM immediate and delayed scores. Secondary endpoints were ADAS-cog word recognition and word recall sub-scores and GDS and BPI.

Statistical analysis

Simple analyses and 95% confidence intervals were calculated using the Minitab Release 13.1. General linear models (Minitab 13.1) for end-treatment ADAS-cog score were constructed with predictors of pre-treatment ADAS-cog score, dummy variable for randomized treatment allocation, and backward selection of clinically relevant potentially confounding variables (PTH, gender, BMI and baseline MMSE). The interaction of insulin/placebo with preceding allocation to high-dose D/placebo high-dose was tested by a general linear model. That model predicted ADAS-cog score after insulin/placebo with the following predictors: ADAS-cog score at start of insulin/placebo, dummy variables for insulin/placebo, preceding high-dose D/placebo, and an interaction term for the preceding high-dose vitamin D/placebo with insulin/placebo. Vitamin D and insulin analyses included all participants who completed randomized treatment. A p value < 0.05 was considered significant.

Power calculations for differences in the median change in ADAS-cog score (treatment versus placebo) were calculated with Minitab 13.1, with alpha 0.05, based on the pilot study standard deviation for change in ADAS-cog score of 3.79 ADAS-cog points. Power calculations for differences in the median change in ADAS-cog score (treatment versus placebo) were calculated with Minitab 13.1, with alpha 0.05, based on the pilot study standard deviation for change in ADAS-cog score of 3.79 ADAS-cog points. Power was 95% for a difference of 5 ADAS-cog points (high-dose vitamin D versus placebo high-dose) and 80% for a difference of 4 ADAS-cog points (insulin versus placebo).

RESULTS

Pilot study

Thirteen individuals with AD, median (interquartile range [IR]) MMSE 21.5 (18.5 to 23), were studied. Median (IR) 25OHD increased from 66 (57–72) to 140 (130–150) nM (P < 0.001). No significant changes occurred in other analytes. Median (IR) baseline ADAS-cog was 25 (20–29) and median (IR) improvement in ADAS-cog score was 6.0 (4.5 to 8.5) points (P < 0.001) (95% confidence interval for improvement in ADAS-cog of 4.7 to 8.3 points). DAD score increased, reflecting less disability, in 11 out of 13 (p < 0.02).

RCT

63 community dwelling individuals with AD were recruited August-December 2006. 29 failed screening and two withdrew after screening (Fig. 1). 32 started treatment (Table 1): 16 took donepezil, 1 rivastigmine, 8 galantamine and 1 galantamine and memantine; 2 had type 2 diabetes treated with oral agents. All completed low-dose vitamin D run-in; 31 completed high-dose D/placebo (Fig. 1), one on placebo, withdrawing after week 6 due to an intercurrent infection. All 31 completed insulin/placebo treatment. No other adverse events were reported.

During low dose run-in median (IR) 25OH-D rose from 49 (39–67) to 60 (55–70) nM (P < 0.01). Median (IR) ADAS-cog, DAD, GDS, and BPI did not change significantly between baseline and 8 weeks being, respectively, 25.5 (20–31) versus 22 (19–30), 77 (56–92) versus 74 (63–92), 2 (1–5) versus 2 (1–3), and 0 (0–1) versus 0 (0–1).

Groups were well matched and, with the exception of PTH (see below), did not differ significantly for any clinical or biochemical parameter in Table 2. Nine women and 7 men were randomized to high-dose, and

<table>
<thead>
<tr>
<th>Feature</th>
<th>Median</th>
<th>Interquartile range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>77.5</td>
<td>60–80</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5</td>
<td>22–26.5</td>
</tr>
<tr>
<td>Years of symptoms</td>
<td>5</td>
<td>3–6.5</td>
</tr>
<tr>
<td>Years since diagnosis</td>
<td>5</td>
<td>2–4.5</td>
</tr>
<tr>
<td>MMSE</td>
<td>19.5</td>
<td>17–22</td>
</tr>
<tr>
<td>ADAS-cog</td>
<td>25.5</td>
<td>20.25–30.75</td>
</tr>
<tr>
<td>WMS-RLM 30 min delayed recall</td>
<td>3.5</td>
<td>2.25–6</td>
</tr>
<tr>
<td>DAD</td>
<td>77</td>
<td>56–92</td>
</tr>
<tr>
<td>GDS</td>
<td>2</td>
<td>1–4.75</td>
</tr>
<tr>
<td>BPI</td>
<td>0</td>
<td>0–1</td>
</tr>
<tr>
<td>25OHD (nM)</td>
<td>49</td>
<td>39–67</td>
</tr>
<tr>
<td>PTH (pM)</td>
<td>4.3</td>
<td>3.2–5.3</td>
</tr>
<tr>
<td>Calcium (mM)</td>
<td>2.46</td>
<td>2.40–2.56</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>43.5</td>
<td>42–45</td>
</tr>
<tr>
<td>Corrected calcium (mM)</td>
<td>2.40</td>
<td>2.34–2.45</td>
</tr>
<tr>
<td>Uric acid (mM)</td>
<td>0.30</td>
<td>0.25–0.34</td>
</tr>
<tr>
<td>Creatinine (mM)</td>
<td>0.09</td>
<td>0.06–0.10</td>
</tr>
</tbody>
</table>
Eight women and 8 men were randomized to insulin, and 9 women and 7 men to placebo. Insulin and placebo groups did not significantly differ for age, BMI, number on AD pharmacotherapy, MMSE, number with MMSE > 22 or any biochemical parameter. No signif-
Fig. 3. Changes in ADAS-cog for the pilot study and randomized trial. Each data point represents the before (X-axis value) and after (Y-axis value) ADAS-cog scores for an individual participant. In the pilot study all points lie to right of the line of identity indicating that for every individual ADAS-cog was lower at study exit. In the randomized trial, participants who received additional high-dose vitamin D2 are plotted as circles and those who received placebo as diamonds. In the RCT, the data points are scattered around the line of identity reflecting a lack of a consistent change in ADAS-cog score.

Table 3

<table>
<thead>
<tr>
<th>Outcome measure</th>
<th>Intervention</th>
<th>95% confidence interval for the difference in median changes across intervention (treatment minus placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAS-cog</td>
<td>High-dose D</td>
<td>-5.0 to 3.0</td>
</tr>
<tr>
<td>DAD</td>
<td>High-dose D</td>
<td>-7.0 to 7.0</td>
</tr>
<tr>
<td>GDS</td>
<td>High-dose D</td>
<td>0 to 2.0</td>
</tr>
<tr>
<td>BPI</td>
<td>High-dose D</td>
<td>0 to 1.0</td>
</tr>
<tr>
<td>WMS-RLM immediate</td>
<td>High-dose D</td>
<td>-1 to 3.0</td>
</tr>
<tr>
<td>WMS-RLM delayed</td>
<td>High-dose D</td>
<td>0 to 1.0</td>
</tr>
<tr>
<td>ADAS-cog</td>
<td>Nasal insulin</td>
<td>-6.0 to 2.0</td>
</tr>
</tbody>
</table>

Significant differences were found between the insulin and placebo groups for any endpoint.

A general linear model predicting change in ADAS-cog score found a significant interaction between insulin/placebo with previous high-dose D/placebo (p = 0.024). However, this was no longer significant (p = 0.144) when the model was extended to take into account the ADAS-cog score at the start of insulin/placebo treatment. In a subgroup analysis, ADAS-cog changed after 48 h in the subgroup that received nasal insulin after placebo high-dose D, with a median [IR] improvement of 9 [1–11] points (p = 0.02, Mann-Whitney). However, WMS-RLM did not change significantly and statistical run-in plots suggested that this 'improvement' in ADAS-cog could have followed a chance worsening and represent regression to the mean.

Post-hoc analysis with general linear modeling restricted to those with MMSE > 22 or according to gender or BMI revealed no benefit from nasal insulin.

DISCUSSION

This is the first double-blinded RCT to test high-dose vitamin D and nasal insulin in AD. Despite rapid improvement in cognition and disability in an open pilot study, the RCT found no benefit from adding high-dose vitamin D to ongoing low-dose vitamin D supplementation. In the RCT, serum 25OHD concentrations were generally supra-physiologic and may have had no additional effect or conceivably a different effect than physiologic 25OHD concentrations in the pilot study. In addition, it is possible that differences in the rates of change of serum 25OHD after high-dose compared to low-dose vitamin D may have influenced the clinical outcome [30]. The RCT was powered to detect the pilot study improvement in ADAS-cog, but a small benefit cannot be excluded. By chance, PTH at the start of the RCT was lower in the high-dose vitamin D group than the placebo group. This may have made it harder to detect a benefit from high-dose vitamin D mediated through suppression of PTH. However, post-hoc analyses considering changes in PTH still did not demonstrate a benefit. The fact that the entire cohort ADAS-cog score was not significantly changed after 16
towards a positive finding but even with post-hoc anal-
lyses that considered severity of underlying dementia,
gender and BMI, we found no benefit from nasal
insulin. A possible limitation is that a pharmacist
supervised only the first nasal dose, subsequent doses
at home being unsupervised. A nasal insulin trial of
longer duration may find different outcomes.

In conclusion, despite reports that dementia is
associated with poor vitamin D nutrition we found
no benefit for cognition or disability from adding
high-dose vitamin D to ongoing low-dose vitamin
D supplementation. Furthermore, despite reports in
unblinded studies of improvement in cognition with
nasal insulin in AD, we found no benefit from nasal
insulin acutely or over 48 h. We cannot exclude the
possibility that an effect of high-dose vitamin D was
obviated by a protective effect of low-dose D. Our
findings will be important for contextualizing previ-
ous correlative studies and for meta-analyses of data
from further controlled studies.

ACKNOWLEDGMENTS

Mr Eric Huggins (Chairman). Dr Peter Habersberger
(Medical Director) and Trustees of The Shepherd
Foundation for generous funding and enthusiastic
support; Dr Mal Eutick (Pharmalab) for advice on
insulin diluent; Alex Kelly (Pharmalab), David Chad-
wick (Pharmatek Fresenius Kab P/L), for preparation
and packaging of diluent; Richard Oppenheim, Hong
Truong (Cardinal Health) for capsule manufacture;
Professor Serge Gaultier, McGill University for per-
mission to use DAD; A/Prof Ian Gordon, Statistical
Consulting Centre, University of Melbourne for ran-
domization and statistical analyses; Lynn Mather,
Alzheimer’s Australia Victoria, Lynne Scott and Eliz-
abeth Fraser for recruitment; Monika Meehan for
pharmacy; Vee Lyn Tan, Susan Edwards, Zoe Ellis,
Caroline Francis, Jennie Barnett, Bianca Duffield
and Shinay Mackey for occupational therapy; Kate
Frencham and Lucy Smith for neuropsychological
assessment; Rhiannon Jones and Karen Harris for
administrative assistance; Maria Bisignano, Cecilia
Hsieh, Max Goodwin and Maraea Harrop for biochem-
ical analyses. LCH is National Health and Medical
Research Council Australia Senior Principal Research
Fellow and is funded by a Victorian State Government
Operational Infrastructure Support Grant.

Authors’ disclosures available online (http://www.j-
REFERENCES


