

Supplementary Online Content

Witham MD, Price RJG, Struthers AD, et al. Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic hypertension: the VitDISH randomized controlled trial. *JAMA Intern Med*. Published online August 12, 2013. doi:10.1001/jamainternmed.2013.9043.

eMethods

eReferences

eTable. Adverse Events

This supplementary material has been provided by the authors to give readers additional information about their work.

Methods

Design and Participants

The study was a randomised, double-blind, placebo controlled, parallel group trial. We studied community dwelling patients aged 70 years with isolated systolic hypertension. Inclusion criteria were: age 70 years and over, serum 25-hydroxyvitamin D (25OHD) level <75 nmol/L; office systolic blood pressure >140 mm Hg. Exclusion criteria were: Diastolic blood pressure >90 mm Hg, systolic blood pressure >180 mm Hg, hypertension known to be due to a correctable underlying medical or surgical cause; estimated glomerular filtration rate <40mls/min (by 4 variable Modified Diet in Renal Disease equation¹); any liver function test (alanine aminotransferase, bilirubin, alkaline phosphatase) >3x upper limit of local normal range; albumin-adjusted serum calcium >2.60 mmol/L or <2.15 mmol/L. We also excluded patients with known metastatic malignancy or sarcoidosis, a history of renal calculi, diagnosis of heart failure with left ventricular systolic dysfunction, atrial fibrillation, and those already taking prescription vitamin D supplements. Consumption of fish oils and low dose (200 IU per day or less) over the counter vitamin D supplements were not a contraindication to enrolment.

Patients were recruited via three routes. The major route was from the community via primary care practices. Patients were identified via General Practice lists of collaborating practices within the East Node of the Scottish Primary Care Research network. First contact was from GPs by letter; those patients expressing interest in the study were put in touch with the research team and were sent study information. Further participants were recruited after responding to an article in the local paper about the research study; a small number of patients were recruited after being approached at secondary care clinics (cardiovascular and Medicine for the Elderly). Research Ethics approval was gained from Fife and Forth Valley NHS Research Ethics Committee (ref: 08/S0501/90). Clinical Trials authorisation was obtained from the UK Medicines and Healthcare Regulatory Authority (EudraCT number 2008-004534-24). The trial sponsor was the University of Dundee, and the trial was registered at www.controlled-trials.com (ISRCTN92186858). The full protocol is available from the authors.

Intervention

Participants entering the study were allocated to intervention or placebo in a 1:1 ratio using a minimisation algorithm, administered by the Robertson Centre for Biostatistics (Glasgow Clinical Trials Unit, University of Glasgow, UK) using a telephone-based system to conceal study allocation from investigators and participants. Minimisation variables used were baseline 25OHD level above or below 50nmol/L, baseline systolic blood pressure above or below 160 mm Hg, baseline age above or below 80 years and presence of diabetes mellitus. Identical, blinded medication bottles were used; patients were observed ingesting 100,000 units of oral vitamin D3 (cholecalciferol) (Vigantol oil, Merck KgAA) or matching placebo (Mygliol oil, Merck KgAA), after completion of baseline, 3, 6 and 9 month assessments.

Outcome measures

All outcomes were performed by researchers who were blinded to treatment allocation. The primary outcome was prespecified as the between-group difference in office blood pressure at 3 months analysed using all available data points regardless of whether participants received their allocated intervention.

Blood pressure measurements were taken at 0, 3, 6, 9 and 12 months using an OMRON HEM-705CP oscillometric machine. After a minimum 5 minute rest, three readings were taken in the supine position, with the mean of the second and third readings taken as the primary measure. Standing blood pressure was then recorded at 0, 1 and 3 minutes.

Secondary outcomes

24 hour blood pressure

24 hour blood pressure was measured using the Meditech ABPM-04 ambulatory monitor at 0, 3, 6, 9, and 12 months. The mean of the 24 hour recording was used as the main measure; day and night time readings were also analysed separately. Sensitivity analyses using a quality threshold (minimum of 14 daytime readings; minimum of 7 nighttime readings as per British Hypertension Society guidance²) were also performed.

Soluble markers of cardiovascular risk

Soluble markers were collected at baseline, 3 and 12 months after a minimum 6 hour fast. Samples for B-type natriuretic peptide (BNP) were collected into EDTA and allowed to stand on ice for 10 minutes, then spun at for 10 minutes. BNP samples were stored at -70°C, and batch analysed at the end of the trial using a radioimmunoassay based kit (Bachem, Merseyside, UK). Intra-assay coefficient of variation for this assay within our laboratory was 14%. High-sensitivity C-reactive protein (hs-CRP) samples were stored at -20°C, and batch analysed at the end of the study using an ELISA assay (Kalon Biologicals, Guildford, UK). Intra-assay coefficient of variation for this assay was 6.1%. Insulin samples were stored at -20°C and batch analysed at the end of the study using a radioimmunoassay kit (Diasorin, Bracknell, UK). Intra-assay coefficient of variation was 5.8%. Insulin and glucose measurements were used to construct a homeostasis model assessment of insulin resistance (HOMA-IR)³ using the formula: fasting glucose (mg/dL) × fasting insulin (uU/mL)/405

Endothelial function

Endothelial function was assessed at 0,3 and 12 months by measuring flow mediated dilatation (FMD) of the brachial artery in response to hyperaemia (endothelium dependent vasodilation) following 5 minutes of forearm cuff occlusion.⁴ An ultrasound system (Sequoia 512, Siemens, Camberley, UK) using an 8MHz linear transducer was used for imaging. Images gated to the ECG R-wave were acquired for 60 seconds before cuff inflation and for 2 minutes after the end of cuff occlusion. Endothelium-independent vasodilatation using sublingual glyceryl trinitrate was not measured for most patients due to an unacceptably high rate of syncopal side effects in this older population during the study (>5%). Artery diameter was measured using Vascular Research Tools software (Medical Imaging Applications LLC, Coralville, IA, USA). Mean diameter was measured during baseline acquisition, and was compared to the maximum diameter achieved after cuff deflation. FMD was then expressed as the percentage change from baseline diameter.

Pulse wave velocity

Carotid-radial pulse wave velocity and augmentation index were measured using the Sphygmocor applanation tonometry system (AtCor Medical, Gloucester, UK) at 0, 3 and 12 months to give indices of arterial stiffness. Measurements were taken in the supine position after a minimum of 5 minutes of rest. For augmentation index, at least 15 good-quality waveforms derived from the radial artery were averaged and transformed to central aortic pressure using the supplied generalised transfer function. Augmentation index is presented normalised to a heart rate of 75 per minute. Carotid and radial tonometry measures were obtained in conjunction with 3 lead ECG monitoring to compare the onset of systole (R wave peak) with the onset of the pulse wave, detected by Sphygmocor software using the intersecting tangents method.⁵ Comparison of delay between R-wave peak and carotid pulse wave onset versus R-wave peak and radial pulse wave onset was used to derive carotid-radial pulse wave velocity.

Other biochemical measurements

We measured glucose, total cholesterol, LDL and HDL cholesterol, triglycerides, serum albumin and calcium on fasting blood samples, analysed using a Roche multichannel analyser system as part of routine NHS biochemistry laboratory workflow. PTH was collected, spun and analysed within 4 hours. 25OHD samples were collected, spun and stored at -20°C and assayed using the IDS ELISA (IDS, Boldon, UK). Intra-assay coefficient of variability was 6.3% and the laboratory participated in the DEQAS quality assurance scheme for vitamin D assay. Screening samples were analysed in small batches prior to entry into the trial to ascertain trial eligibility; follow-up samples were stored and batch analysed at the end of the trial.

Asymptomatic hypercalcaemia (adjusted serum calcium >2.60mmol/L) was recorded as an adverse outcome; no further doses of study medication were administered if this occurred, but follow-up visits for outcomes continued. All adverse events were recorded at each study visit, with specific questioning regarding nausea, vomiting, diarrhoea and new loin pain. Information on usual activity was collected along with medications and comorbid disease. Calcium intake was assessed at baseline by the Scottish Collaborative Group Food Frequency Questionnaire.⁶

Exercise capacity and falls

Exercise capacity was determined by six minute walk at 0, 6 and 12 months.⁷ The walk test was performed on a 25 metre flat course, using standardised encouragement every 2 minutes. Falls frequency was prospectively recorded by patients using monthly diaries.

Statistical analysis

Analyses were performed by the Robertson Centre for Biostatistics using R v2.15.0 (R Foundation, Vienna, Austria).⁸ All evaluable datapoints were included, regardless of whether participants received the allocated intervention or not. A 2 sided p value of <0.05 was taken as significant for all analyses. For each outcome, the between group difference at each timepoint was calculated. In addition, repeated measures ANOVA was undertaken to give an estimate of overall treatment effect across time.

Preplanned adjustment for covariates (ANCOVA) was undertaken for all outcomes. Adjustment was made for baseline values of the outcome under study, along with baseline vitamin D, baseline blood pressure, diabetes and age. We also adjusted for use of thiazide diuretics given the known effects of these medications on calcium metabolism. Similarly, use of statins was included as a covariate. Sensitivity analyses were conducted using multiple imputation to account for missing data, and excluding patients with changes in antihypertensive medication for blood pressure analyses.

Sample size calculation

Based on data from a previous vitamin D intervention trial for hypertension,⁹ we powered the study to detect a 7 mm Hg between-group difference in systolic blood pressure change between baseline and 3 months, assuming a standard deviation of change of 18 mm Hg. To detect this change with 80% power at an alpha level of 0.05 required 73 patients per group. We originally anticipated a dropout rate of 20%, and thus aimed to recruit 180 patients into the trial.

eReferences

1. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med.* 1999; 130(6):461-470.
2. O'Brien E, Coats A, Owens P et al. Use and interpretation of ambulatory blood pressure monitoring: recommendations of the British hypertension society. *BMJ.* 2000;320(7242):1128-1134.
3. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-419.
4. Corretti MC, Anderson TJ, Benjamin EJ et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. *J Am Coll Cardiol.* 2002;39(2):257-265.
5. Wilkinson IB, Fuchs SA, Jansen IM et al. Reproducibility of pulse wave velocity and augmentation index measured by pulse wave analysis. *J Hypertens.* 1998;16(12 pt 2):2079-2084.
6. Masson LF, McNeill G, Tomany JO et al. Statistical approaches for assessing the relative validity of a food-frequency questionnaire: use of correlation coefficients and the kappa statistic. *Public Health Nutr.* 2003;6(3):313-321.
7. Perera S, Mody SH, Woodman RC, Studenski SA. Meaningful change and responsiveness in common physical performance measures in older adults. *J Am Geriatr Soc.* 2006;54(5):743-749.
8. R Development Core Team (2012). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org/>.

9. Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. *Diabetologia*. 2010;53(10):2112-2119.

eTable. Adverse Events

Adverse event	Vitamin D (n=80)	Placebo (n=79)	<i>P</i> value
Nausea/vomiting	35	26	.31
Constipation	13	13	>.99
Diarrhea	16	22	.33
Dyspepsia	7	8	.80
Loin pain/dysuria	4	3	>.99
Coronary vascular event ^a	2	2	>.99
Infection	17	25	.22
New malignancy	2	4	.45
Stroke/transient ischaemic attack	3	1	.63
All adverse events	220	235	.43
Deaths	0	1	.50

^aMyocardial infarction, acute coronary syndrome or coronary revascularisation procedure