Effects of egg consumption on the vitamin D status of adults

Final Project Report | **February 2022**

A report for Australian Eggs Limited
by R.M. Daly and S.Y. Tan

Australian Eggs Limited Publication No. 1NM002

ISBN 978-1-920835-74-3

Project Title: Effects of egg consumption on the vitamin D status of adults

Australian Eggs Limited Project Number 1NM002

Researcher/Author Contact Details

Name:	Prof Robin M Daly
Address:	Deakin University Institute for Physical Activity and Nutrition (IPAN) 221 Burwood Hwy Burwood VIC 3125
Phone:	03 9244 6040
Fax:	N/A
Email:	rmdaly@deakin.edu.au

In submitting this report, the researcher has agreed to Australian Eggs Limited publishing this material in its edited form.

Australian Eggs Limited Contact Details:

Australian Eggs Limited
A.B.N: 66 102 859 585
Suite 6.02, Level 6, 132 Arthur St
North Sydney NSW 2060

Phone: 02 9409 6999
Fax: 02 9954 3133
Email: research@australianeggs.org.au
Website: www.australianeggs.org.au

Published in February 2022

Foreword

Vitamin D deficiency is a common public health problem globally. Circulating 25-hydroxyvitamin D [25(OH)D] concentration is considered the best biomarker of vitamin D status. In Australia, findings from the 2011–12 National Health Survey revealed that young adults were at greatest risk of having low 25(OH)D concentrations, especially during the winter months when sun exposure is low. Eggs are one of the few naturally rich food sources of vitamin D, but whether they can prevent the wintertime decrease in blood vitamin D levels is uncertain. This 12-week intervention trial in young adults was conducted to assess the impact of consuming 7 eggs/week (the current amount recommended in the Australian Dietary Guidelines) or 12 eggs/week, compared to a control intake of 2 eggs/week, on blood vitamin D [25(OH)D] concentrations. We conducted a three-arm randomised controlled trial to determine the potential dose-response relationship between egg consumption and 25(OH)D concentrations. Our overall aim was to determine whether there is an optimal dose of eggs to maintain (or increase) vitamin D concentrations in young adults during the winter months. In addition, this study also investigated the effects of the intervention on blood lipids, and the feasibility and acceptability of consuming the number of eggs used in this study. Our findings will help to inform whether increasing egg consumption is an effective approach to maintain (or increase) vitamin D concentrations during the winter-spring months in Australia.

This project was funded from industry revenue, which is matched by funds provided by the Australian Government.

This report is an addition to Australian Eggs Limited's range of peer reviewed research publications and an output of our R&D program, which aims to support improved efficiency, sustainability, product quality, education and technology transfer in the Australian egg industry.

Most of our publications are available for viewing or downloading through our website:

www.australianeggs.org.au

Printed copies of this report are available for a nominal postage and handling fee and can be requested by phoning (02) 9409 6999 or emailing research@australianeggs.org.au.

Acknowledgments

Australian Eggs Limited provided the funds that supported this project.

We wish to thank Dr Zhong Lu from Monash Pathology, Monash Health for overseeing the assessment of blood 25-hydroxyvitamin D concentrations and the research participants for their time and effort.

About the Authors

Principal Investigator – Professor Robin M Daly

Professor Daly (PhD, FASMF, FASBMR) holds the position of Chair in Exercise and Ageing and co-leads the Preventing and Managing Chronic Diseases domain within the Institute for Physical Activity and Nutrition (IPAN) at Deakin University. His research is focused on the integration and implementation of nutrition and exercise strategies to prevent and manage chronic diseases such as osteoporosis, sarcopenia, falls/fractures, type 2 diabetes, certain cancers, and cognitive related disorders. He has over 25 years of experience in conducting clinical and community-based nutrition and exercise trials across multiple populations (children, middle-aged adults and the elderly, and those with chronic conditions). His research expertise and interests also include health issues related to vitamin D deficiency (and treatment), dietary protein, sedentary behaviour and chronic inflammation, and the translation of research into practice. He has been an active contributor nationally and internationally to clinical guidelines in the areas of exercise, dietary calcium and vitamin D.

Co-investigator – Dr Sze Yen Tan

Dr Tan is a Senior Lecturer and the Deputy Course Director for the Bachelor of Nutrition Science at Deakin University. He is also an active mid-career researcher (10 years post-PhD) working in the Institute for Physical Activity and Nutrition (IPAN). He is an Advanced Accredited Practising Dietitian (AdvAPD) and a Registered Nutritionist (RNutr) who has completed both acute and long-term human studies ranging from a few hours using an acute feeding study design to 12 months. Dr Tan's research focuses on finding effective dietary strategies that promote optimal nutritional status and health in adults. His research examines dietary strategies such as the manipulation of nutrients, e.g. protein and polyunsaturated fats, and how these translate into whole food or dietary approaches to promote optimal health and disease prevention or management.

Table of Contents

List of Tables

List of Figures

Abbreviations

25(OH)D	25-hydroxyvitamin D
AE	Adverse events
AI	Adequate intake
ANCOVA	Analysis of covariance
AUSNUT	Australian Food and Nutrient Database
CI	Confidence interval
CV	Cardiovascular
d	Day
FAQ	Food Acceptability Questionnaire
g	Gram
HDL	High-density lipoprotein
HPLC PDA	High performance liquid chromatography with photodiode array
IU	International unit
Kcal	Kilocalories
LC/MS/MS	Liquid chromatography with two mass spectrometry detectors
LDL	Low-density lipoprotein
µg/d	Micrograms per day
min	Minutes
mmol/l	Millimoles per litre
MVPA	Moderate-vigorous physical activity
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
NMI	National Measurement Institute of Australia
nmol/L	Nanomoles per litre
RCT	Randomised controlled trial
SD	Standard deviation
USA	Unites States of America
UV	Ultraviolet

Executive Summary

Vitamin D deficiency, defined as a low circulating 25(OH)D concentration, is a common problem globally, particularly during winter at higher latitudes. Eggs are one of the few naturally rich food sources of vitamin D, containing both vitamin D_3 and 25-hydroxyvitamin D_3 [$25(OH)D_3$], with the latter reported to be five times more potent at increasing blood 25(OH)D concentrations. However, whether there is an optimal dose of eggs to increase circulating 25(OH)D concentrations or prevent the wintertime decline remains uncertain. The aim of this study was to evaluate the dose-response effect of consuming 2 (control condition), 7 or 12 commercially available eggs/week on serum 25(OH)D concentrations during the autumn-winter months in young Australian adults aged 25–40 years. Secondary aims were to investigate the effects of the intervention on blood lipids, and the feasibility (adherence to the different egg doses) and acceptability to consuming the different doses of eggs. In this 12-week, randomised controlled trial, 51 adults aged 25–40 years residing in Melbourne and Geelong, Australia were randomised to consume either 2 eggs/week (control, n = 17), 7 eggs/week (n = 17) or 12 eggs/week (n = 17). Serum 25(OH)D was the primary outcome as assessed by the gold standard method (LC/MS/MS). Blood lipids were assessed using standard techniques, and acceptability of eating eggs through questionnaire. Forty-two (82%) participants completed the study. Mean adherence to the eggs was 83% for controls, 86% for 7 eggs/week and 83% for 12 eggs/week. Mean (95% CI) serum 25(OH)D concentrations did not change significantly after 12 weeks in either the 7 eggs/week [-8.3 (-17.0, 0.4) nmol/L] or 12 eggs/week [-7.2 (-18.6, 4.3) nmol/L] group, but decreased by 28.6 nmol/L (-38.1, -18.9) in controls, which led to a significant (P = 0.003) between-group difference for the change after 12 weeks. Blood lipids did not differ between the groups, and acceptability profiles to consuming the eggs were positive and similar for all three groups. In conclusion, consuming 7 commercially available eggs/week for 12 weeks was effective for attenuating the wintertime decline in circulating vitamin D concentrations in young Australian adults, with consumption of 12 eggs/week not conferring any additional benefits.

Overall Conclusions

- Consumption of 7 commercially available eggs per week, which is in line with the currentAustralian dietary guidelines, was safe, acceptable, and effective at attenuating the wintertime decline in serum 25(OH)D concentrations in young Australian adults.

- Consuming 12 eggs per week did not confer any added benefits on serum 25(OH)D concentrations over 7 eggs per week.

- Participant acceptability profiles to consuming the eggs were positive for all groups, and there were no adverse effects of consuming the eggs on body weight or blood lipid concentrations.

- Overall, the findings from this study indicate that weekly consumption of commercially available 7 eggs should be considered as an important dietary approach to help to optimise vitamin D status, especially during the winter months in Australia and in people that might receive limited sun exposure (e.g. office and night shift workers).

1 Background

Vitamin D deficiency is purported to be a global public health problem (Hilger et al. 2014; Amrein et al. 2020). In Australia, the 2011–12 National Health Survey revealed that 20% of adults (~3.29 million people based on census data) aged ≥ 25 years were vitamin D deficient (25-hydroxyvitamin D < 50 nmol/L), and a further 43% were classified as having insufficient concentrations (below 75 nmol/L), with the highest rates of deficiency in adults aged 25–34 years (Malacova et al. 2019). This is likely due in part to the greater use of vitamin D-containing supplements in older Australian adults (Black et al. 2016). Given the importance of vitamin D to bone health and possibly other non-skeletal conditions, strategies are needed to maintain adequate 25(OH)D vitamin D concentrations, particularly in young adults during winter when serum 25(OH)D concentrations decrease by approximately 15–20 nmol/L and the risk of low 25(OH)D more than doubles (Daly et al. 2012; Malacova et al. 2019).

The main source of vitamin D is through sun exposure, but due to geographical differences, seasonal changes in ultraviolet (UV) radiation, differences in skin pigmentation, lifestyle habits and adherence to sun protection messages, it can be difficult to achieve adequate year-round exposure to maintain sufficient serum 25(OH)D concentrations. In addition, since few foods naturally contain vitamin D many adults have habitual intakes below the current NHMRC adequate intake (AI) guidelines, which range from 5 to 15 µg/d increasing with age (Nowson et al. 2012; National Health and Medical Research Council (NMHRC) 2016; Ross et al. 2011) or up to 20 µg/d (800 IU/d) for adults aged > 70 years (Ross et al. 2011). Of the limited food sources that naturally contain vitamin D (e.g. oily fish, meat, eggs, dairy), eggs have significant quantities of both vitamin D_3 and the hydroxylated form of vitamin D_3, 25(OH)D_3, which may be up to five times more potent in raising serum 25(OH)D concentrations than vitamin D_3 (Cashman et al. 2012; Ovesen et al. 2003). As a result, there has been interest in whether regular consumption of eggs can play a key role in maintaining circulating 25(OH)D concentrations, but the optimal dose required to increase or maintain 25(OH)D concentrations is not known.

Several studies measuring the vitamin D concentrations of Australian eggs have reported the vitamin D_3 and 25(OH)D_3 content (free range and cage eggs) per egg (60 g) to be 0.4 to 0.8 µg and 0.4 to 0.6 µg, respectively (Dunlop et al. 2017; Dunlop et al. 2021). Assuming 25(OH)D_3 is five times more bioactive than vitamin D_3, which is a correction factor included in some vitamin D food composition databases to provide a more accurate estimate of vitamin D intakes, the total vitamin D activity of eggs would be 2.5 to 3.4 µg per egg. Based on a standard 120 g serve of eggs (2 large eggs), this dose would be equivalent to the current AI of 5 µg/day for vitamin D for Australians aged 1–50 years (National Health and Medical Research Council (NMHRC) 2016). Previous research has shown that serum 25(OH)D concentrations increase by ~1-2 nmol/L for every additional 2.5 µg of vitamin D_3 (Cranney et al. 2007; Nikooyeh & Neyestani 2021; Black et al. 2012). Since the current Australian Dietary Guidelines recommend eating up to seven eggs/week as part of a healthy, balanced diet (NHMRC 2013), it is feasible that this weekly dose would be sufficient to maintain or attenuate the wintertime decrease in serum 25(OH)D concentrations. Indeed, the findings from an 8-week randomised controlled trial in 55 adults aged 45–70 years residing in Ireland with habitual vitamin D intakes of ~6.0-6.9 µg/d revealed that weekly consumption of seven vitamin D_3-enriched eggs or seven 25(OH)D-enriched eggs (providing an additional 3.5 and 4.5 µg/egg – total vitamin D activity) were equally effective at maintaining serum 25(OH)D concentrations compared to a low egg group (consuming ≤ 2 eggs/week) who experienced a significant mean 6.4 nmol/L reduction in circulating 25(OH)D concentrations (Hayes et al. 2016). While these findings provide some evidence to support the weekly consumption of seven vitamin D-fortified eggs for maintaining wintertime vitamin D status, no studies have investigated whether there is a dose-response relationship between egg consumption and serum 25(OH)D concentrations. This is important to determine whether there may be a minimum dose of eggs that might be required to maintain or increase circulating 25(OHD) concentrations.

The primary aim of this 12-week randomised controlled trial was to compare the effects of consuming 2, 7 and 12 commercially available eggs/week on serum 25(OH)D concentrations during the autumn-winter months in adults aged 25–40 years residing in southern Australia (latitude ~38°S). Secondary aims were to investigate the effects of the intervention on blood lipids, as there has been some concern that eggs might increase the risk of cardiovascular disease, and the feasibility (adherence) and acceptability to consuming the different doses of eggs.

2 Subjects and methods

2.1 Study design

This was a 12-week, three-arm, randomised controlled trial (RCT) in which 51 men and women aged 25–40 years were randomly allocated (1:1:1 ratio) to consume: 1) 2 eggs/week (control group, n = 17); 2) 7 eggs/week (n = 17); or 3) 12 eggs/week (n = 17). Randomisation was at the level of the individual participant in blocks of three using a computer-generated random number sequence by an independent researcher. The intervention was conducted from May (late autumn) to August (winter) 2021 in Melbourne, Australia. All baseline and follow-up assessments were performed at a local pathology clinic (blood collection) or online (questionnaires), and participants and the research staff conducting the trial were not blinded to the group allocation. The trial was managed through the Institute for Physical Activity and Nutrition at Deakin University, Burwood, Melbourne, Australia.

2.2 Participants

Healthy men and women aged 25 to 40 years residing in the community were recruited from metropolitan Melbourne and Geelong (latitude ~38°S) in Victoria, Australia. Interested participants were first screened via an online survey in which they progressed to stage 2 (telephone screening) if they were aged 25–40 years, not allergic to eggs, a non-smoker, had an occupation or lifestyle over the past 3 months that involved spending on average < 3 hours per day outdoors between 10am and 2pm, and were currently not taking (or in the past 3 months) vitamin D supplements or multivitamin supplements containing a vitamin D dose > 200 IU. Eligible participants were then contacted by the research staff and deemed ineligible based on the following: regular consumers of whole eggs (> 3 per week on average over the past 3 months); currently participating in a weight loss or dietary-based program; currently taking corticosteroids (oral), glucocorticoids, anticonvulsants or thiazide diuretics; current diagnosis of cancer (or in past 6 months), diabetes, kidney disease or a gastrointestinal disorder that can affect nutrient absorption; a cardiovascular (CV) event that required hospitalisation in the past3 months, or currently undergoing CV rehabilitation; alcohol intake > 2 standard drinks on five or more days per week; recent (past 3 months) and proposed holiday or trip (> 1 week) involving a high level (more than usual) of sun exposure; use of tanning facilities in the past 3 months; unable to commit to the study requirements; and not willing to be randomised to one of the three groups.

A total of 361 adults expressed an interest in participating in the study, of which 51 were deemed eligible to participate and randomised into one of the three groups. The study was approved by the Deakin University Human Research Ethics Committee (HREC 2020-159) and was registered with the Australian and New Zealand Clinical Trials Registry (ACTRN12620001057976). Written informed consent was obtained from all participants prior to commencing the study.

2.3 Intervention

Participants were randomised to one of three groups: 1) a control group in which they were asked to consume 2 eggs/week; 2) consumption of 7 eggs/week in line with the current Australian dietary guidelines; or 3) consumption of 12 eggs/week. Due to COVID restrictions on travel, all participants were provided with a pre-paid gift card and asked to purchase a 3-week supply of the same brand and size of eggs from the same major supermarket chain (Woolworths Extra Large Free Range Eggs, ~60 g/egg) several days prior to starting the study and during weeks 3, 6 and 9. All participants were required to send an electronic version (photo) of their receipt to confirm purchase of the correct type and dose of eggs. They were encouraged to eat the eggs in whole form where possible (e.g. boiled, scrambled, poached, fried) and not as part of shared meals, not to consume any non-study eggs, and

to maintain their typical diet (eating) habits throughout the study. Adherence to the eggs was determined from a daily eggs compliance calendar that was placed on the participants' fridges via a magnet strip. Overall egg compliance was calculated as the percentage of the eggs consumed relative to the total prescribed.

2.4 Assessment of the vitamin D content of the eggs

The vitamin D_3 and 25(OH)D_3 content of the commercially available eggs was assessed at baseline and week 6 at the National Measurement Institute of Australia (NMI), Port Melbourne, Victoria, which is accredited by the National Association of Testing Authorities (NATA) for analysing vitamin D in foods. At each timepoint, three batches of free range eggs (60 g/egg) purchased from three different supermarkets (from the same major chain – maximum 10,000 birds per hectare stocking density) in the north, east and west of Melbourne were assessed to reflect the broad location (place of residence) of the participants enrolled in the study. The vitamin D_3 and 25(OH)D_3 content of the eggs was assessed by high performance liquid chromatography with photodiode array (HPLC PDA) (De Leenheer et al. 1985). The vitamin D content (expressed as µg per 60 g egg) of each batch of eggs was averaged to give a mean (±SD) value of vitamin D_3 and 25(OH)D_3 at baseline and week 6. Since previous research has shown that each microgram of orally consumed 25(OH)D_3 was five times more effective in raising serum 25(OH)D concentrations that an equivalent amount of vitamin D_3 (Cashman et al. 2012), the total vitamin D content of the eggs (expressed as both µg/egg and IU/egg) was calculated as follows: vitamin D_3 + [5 x 25(OH)D_3].

2.5 Serum 25(OH)D and blood lipids

Fasted, resting morning venous blood samples were collected at baseline and post-intervention from each participant's antecubital vein at local pathology clinics around Melbourne, and sent to a central NATA-accredited pathology laboratory for processing. Total serum cholesterol, high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol and triglycerides were assessed using standardised techniques. Serum 25(OH)D was assessed using the gold standard and validated LC/MS/MS method. All samples were assessed in the same batch (in duplicate) at the completion of the study.

2.6 Demographic, anthropometric, medical history, skin type andadverse events

Height and weight were self-reported as participants were unable to attend Deakin University due to COVID restrictions. Information on demographics (age, gender, ethnic status, educational background), use of lipid lowering or other medications known to influence vitamin D metabolism (e.g. antibiotics, anticonvulsants), skin type (Fitzpatrick skin type scale I–VI), and dietary supplement use was obtained from a health and lifestyle questionnaire. Participants were asked to report any alterations to, or new, medications or use of supplements at follow-up. Any adverse events (AEs) associated with eating the eggs were determined via an online questionnaire that the participants completed every 3 weeks.

2.7 Diet and physical activity

Nutrient intakes were assessed at baseline and follow-up from two 24-hour food records (one weekday and one weekend day), using the Automated Self-Administered 24-Hour Dietary Assessment Tool (ASA24®, version Australia 2016). An assessment of dietary vitamin D intake was not possible as vitamin D values are not included in the AUSNUT 2011–13 database. Self-reported time spent in moderate-vigorous physical activity (MVPA) was assessed at baseline and follow-up using the Active

Australia survey (Heesch et al. 2011; Brown et al. 2008). Total MVPA (minutes per week) was computed as the sum of walking, moderate and vigorous physical activity.

2.8 Sun exposure and protection practices

To provide an estimate of sun exposure habits throughout the study, participants were asked at baseline and every 3 weeks throughout the study (via an online questionnaire) about the time they usually spent outdoors between 10am and 2pm [the peak UV period during May to August in Melbourne] on both weekdays and weekend days in the past 3 weeks. Participants were also asked about their sun protection practices when outdoors, including use/wearing of a broad-brimmed hat, cap or other head covering, wearing of a long sleeve shirt and long pants, and use of sunscreen.

2.9 Egg acceptability

Egg consumption acceptability was evaluated at the end of the study using a modified version of the Food Acceptability Questionnaire (FAQ) (Barnard et al. 2009). For this study, the questionnaire consisted of 7 questions scored on a 7-point response scale in which participants were asked to rate whether they liked eating the eggs, whether they liked the taste of eggs, how satisfied they felt after eating the eggs, how easy/difficult it was for them to prepare the eggs to eat, the level of effort required to eat the eggs, whether eating out influenced regular egg consumption, and the ease at which they could continue to eat the same number of prescribed eggs after completion of the study.

2.10 Sample size

The number of participants required for the study was based on the expected difference for the change in serum 25(OH)D concentrations between the 7 and 12 eggs/week group relative to controls (2 eggs/week). This was based on the findings from Hayes et al. (2016) who reported that consumption of 7 eggs/week (containing 3.5–4.5 µg per egg) maintained serum 25(OH)D concentrations during wintertime compared to controls who experienced a mean (SD) 6.4 (6.7) nmol/L decrease after 8 weeks. Our previous findings from a 16-week study in middle-aged women followed during the winter months in Melbourne (Australia) also found that serum 25(OH)D concentrations decreased by an average of 12 nmol/L after 4 months (Daly et al. 2020). Based on these findings, we estimated that 51 participants (17 per group) would provide 90% power (two-tailed, $P < 0.05$) to detect an 8 and 14 nmol/L net difference between the control (2 eggs/week) and 7 and 12 eggs/week group, respectively, using a conservative standard deviation (SD) of 10. These sample size calculations take into account a potential 20% attrition after 12 weeks.

2.11 Statistical analysis

All statistical analysis was conducted using SPSS for Windows (version 26; SPSS Inc). All data were analysed using an intention-to-treat approach, with every randomised participant included in the analyses. Sensitivity analysis (per protocol) was also performed by only including participants with ≥ 80% adherence to the eggs. Normality of data was tested via visual inspection of frequency distributions and the use of the Kolmogorov-Smirnov tests. All serum lipid measures were log transformed due to non-normality. Descriptive statistics are reported as mean ± standard deviations (SD) or number with proportion (percentage), and all change data are reported as means with 95% confidence intervals, unless otherwise stated. Analysis of covariance (ANCOVA) was used to test for between-group differences for the absolute changes over 12 weeks, adjusting for the baseline variable being tested, age and sex, with Bonferroni-adjusted t-tests used for post-hoc analysis. For the primary outcome of serum 25(OH)D, additional analysis was performed that also included country of birth, physical activity and sun exposure as covariates. Sensitivity analysis was also undertaken excluding

any participants who commenced taking vitamin D supplements during the study for > 1 week. Paired t-tests were used to test for within-group changes over time. For any missing data, no imputation was performed. Chi-squared tests were used to test for group differences in the responses to the egg acceptability questions, with results reported as median and interquartile range. Statistical significance was set at $P < 0.05$.

3 Results

3.1 Vitamin D content of the eggs

The mean ± SD vitamin D content of the eggs (per 60 g egg) used throughout the study was 0.71 ± 0.25 µg/egg for 25(OH)D_3, 1.63 ± 0.70 µg/egg for cholecalciferol, and 5.18 ± 1.60 µg/egg for total vitamin D (Table 1). Based on the total vitamin D concentrations of the eggs, the prescribed weekly vitamin D dose for the control (2 eggs/week), 7 and 12 eggs/week groups were 10.4 µg (414 IU), 36.3 µg (1450 IU) and 62.2 µg (2486 IU), respectively.

Table 1 Vitamin D content of the commercial eggs used in the study[1]

	Vitamin D content of eggs (µg per 60 g egg)		
	Vitamin D_3	**25(OH)D_3**	**Total vitamin D activity[2]**
Baseline	1.82 ± 0.61	0.72 ± 0.16	5.42 ± 0.64
Week 6	1.44 ± 0.87	0.70 ± 0.37	4.94 ± 2.42
Mean	*1.63 ± 0.70*	*0.71 ± 0.25*	*5.18 ± 1.60*

[1] Values are means ± standard deviations.

[2] Total vitamin activity was derived from the following formula: vitamin D_3 + [5 x 25(OH)D_3].

3.2 Baseline characteristics of study participants

As shown in Table 2, the average age of the participants was ~33 years, 75% were female, 41% were overweight or obese, 71% were classified as Europid and the vast majority (86%) had a serum 25(OH)D concentration ≥50 nmol/L at baseline (mean 79 nmol/L). No participants were taking lipid lowering medication or other medication(s) known to influence vitamin D metabolism.

Table 2 Baseline characteristics of the three groups[1]

Characteristic	Control	7 eggs/week	12 eggs/week
N	17	17	17
Men | Women, *n (%)*	3 | 14	5 | 12	5 | 12
Age (years)	33.4 ± 5.4	32.7 ± 5.0	32.7 ± 5.5
Height (cm)	165.0 ± 10.1	168.5 ± 10.8	169.7 ± 9.8
Weight (kg)	67.0 ± 12.5	76.1 ± 25.4	71.5 ± 15.4
BMI (kg/m^2)	24.5 ± 3.9	26.6 ± 8.2	24.8 ± 4.6
Healthy (BMI 18-<25), n (%)	10 (59%)	10 (59%)	10 (59%)
Overweight (BMI 25-29.9), n (%)	6 (35%)	5 (29%)	5 (29%)
Obese (BMI ≥ 30), n (%)	1 (6%)	2 (12%)	2 (12%)
Country of birth, n (%)			
Europid	12 (71%)	11 (65%)	13 (77%)
Non-Europid	5 (29%)	6 (35%)	4 (23%)
Skin type[2], n (%)			
Type I-II	11 (65%)	6 (35%)	10 (59%)
Type III-IV	6 (35%)	10 (59%)	7 (41%)
Type V-VI	0 (0%)	1 (6%)	0 (0%)
Highest level of education, n (%)			
High school or Trade certificate	4 (23%)	1 (6%)	3 (18%)
University or higher	13 (77%)	16 (94%)	14 (82%)
Dyslipidemia[3] , n (%)	3 (18%)	2 (12%)	4 (24%)
Vitamin D status			
Insufficient (<75 nmol/L), n (%)	10 (59%)	8 (47%)	9 (53%)
Deficient (<50 nmol/L), n (%)	1 (6%)	4 (24%)	2 (12%)

[1] Values represent number and percentage or mean ± standard deviations (SD). BMI, body mass index.
[2] Fitzpatrick skin type scale:
Type I: pale white skin, always burns, never tans;
Type II: white or fair skin, usually burns, tans minimally;
Type III: light brown skin, sometimes mild burn, tans uniformly;
Type IV: moderate brown skin, rarely burns, always tans well;
Type V: dark brown, moderately pigmented brown skin, very rarely burns, tans very easily;
Type VI: black deeply pigmented dark brown to black skin; never burns, tans very easily.
[3] Dyslipidemia, cholesterol ≥ 5.5 mmol/L or LDL-cholesterol ≥ 3.5 mmol/L, or HDL-cholesterol < 1.0 mmol/L or triglycerides ≥ 2.0 mmol/L.

3.3 Study attrition, egg adherence and adverse events

Overall, 9 (18%) of the participants did not complete the 12-week follow-up assessment (controls, n = 4; 7 egg/week, n = 3; 12 eggs/week, n = 2). The reasons for withdrawal or lack of follow-up included: lost contact (n = 8) or withdrew with no reason stated (n = 1). Participants with no follow-up assessment did not differ significantly in age, height, weight, physical activity, serum 25(OH)D or blood

lipids from those who completed the intervention (data not shown). The mean (95% CI) adherence to the eggs for all participants was 84% (75, 93) and did not differ significantly (P = 0.94) between the control [mean (95%CI): 83% (65, 100)], 7 eggs/week [86% (71, 100)] and 12 eggs/week [83% (66, 100)] groups. Five participants (n = 2, controls; n = 1, 7 eggs/week; n = 2, 12 eggs/week) did not consume any eggs and all did not complete the 12-week follow-up assessment. For the 42 participants that completed the study, mean adherence to the eggs was 96 ± 9% (range 54, 100) and similar between the groups: controls 98%; 7 eggs/week 98%; 12 eggs/week 94%. One minor adverse event (gastrointestinal discomfort) was reported by one participant in the 12 eggs/week group.

3.4 Weight, physical activity, diet and supplement use

There were no within-group changes nor between-group differences for weight, MVPA or diet throughout the study, with the exception that total protein intake increased on average, by 14 g (P < 0.05) in the 12 eggs/week group (Table 3). At baseline and follow-up, 37% and 38% of all participants reported consuming alcohol (mean intake 19 g and 18 g at baseline and follow-up, respectively), with no differences between the groups (baseline, P = 0.485; week 12, P = 0.729). One participant from both the 7 and 12 eggs/week group reported taking vitamin D supplements during the study (7 eggs/week, 200 IU from week 2; 12 eggs/week, 1000 IU from week 4).

Table 3 Mean dietary intakes and moderate-vigorous physical activity levels at baseline and week 12 in the control, 7 and 12 eggs per week groups[1]

	n	Control	n	7 eggs/week	n	12 eggs/week	P-value[3]
Energy, Kcal/d							
Baseline	16	7574 ± 1902	16	8194 ± 1787	17	9031 ± 2700	*0.638*
Week 12	11	7236 ± 1958	13	8364 ± 2033	13	8918 ± 2073	
P-value [2]		*P=0.353*		*P=0.896*		*P=0.232*	
Protein, g/d							
Baseline	16	76.9 ± 20.1	16	85.9 ± 29.7	17	81.0 ± 24.2	*0.213*
Week 12	11	75.7 ± 33.2	13	103.9 ± 31.3	13	100.1 ± 24.7	
P-value [2]		*P=0.846*		*P=0.187*		*P=0.029*	
Carbohydrates, g/d							
Baseline	16	194.3 ± 57.5	16	196.1 ± 40.3	17	245.6 ± 98.4	*0.844*
Week 12	11	175.1 ± 48.6	13	194.6 ± 40.3	13	211.0 ± 66.8	
P-value [2]		*P=0.250*		*P=0.736*		*P=0.055*	
Fat, g/d							
Baseline	16	72.8 ± 24.7	16	78.3 ± 23.6	17	84.7 ± 31.9	*0.773*
Week 12	11	73.6 ± 30.3	13	77.1 ± 30.8	13	87.7 ± 26.1	
P-value [2]		*P=0.692*		*P=0.728*		*P=0.803*	
Saturated fat, g/d							
Baseline	16	25.4 ± 10.2	16	27.7 ± 9.7	17	28.2 ± 12.3	*0.335*
Week 12	11	29.1 ± 14.6	13	24.5 ± 10.3	13	31.5 ± 13.8	
P-value [2]		*P=0.291*		*P=0.295*		*P=0.969*	
Physical activity, min/week							
Baseline	17	325 ± 178	17	379 ± 300	17	430 ± 374	*0.392*
Week 12	14	238 ± 230	13	393 ± 321	15	347 ± 305	
P-value [2]		*P=0.382*		*P=0.500*		*P=0.531*	

[1] All values are unadjusted means ± standard deviations (SD).

[2] Paired t-tests used to test for differences between baseline and week 12 values.

[3] P-values for between-group differences assessed using ANCOVA adjusted for baseline values, age and sex.

3.5 Serum 25(OH)D concentrations

Mean serum 25(OH)D concentrations decreased non-significantly after 12 weeks by 8.3 nmol/L (P = 0.061) and 7.2 nmol/L (P = 0.20) in the 7 and 12 eggs/week group, respectively. In contrast, in the controls there was a significant 29 nmol/L mean reduction (P < 0.001) in 25(OH)D concentrations after 12 weeks, which led to a significant (P = 0.003) between-group difference for the change after 12 weeks (Table 4 and Figure 1). Post-hoc analysis revealed that changes in the controls were significantly different from both the 7 and 12 eggs/week group, in which the changes were no different between these two groups (Table 4 and Figure 1). All results remained unchanged after also including ethnicity, physical activity and sun exposure as covariates (P = 0.005) or excluding the two participants who commenced taking vitamin D supplements during the trial (P = 0.002), with the exception that mean serum 25(OH)D concentrations decreased significantly from baseline to 12 weeks in both the 7 eggs/week (mean change, 9.7 nmol/L, P = 0.036) and 12 eggs/week (10.7 nmol/L, P = 0.027) groups.

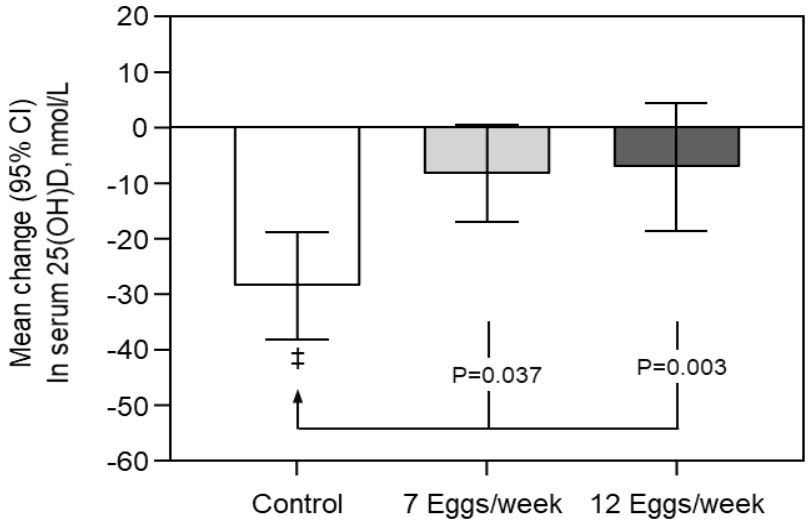

Figure 1 Unadjusted absolute changes in serum 25(OH)D concentrations in young adults after 12 weeks of consuming 2 (control) eggs/week, 7 or 12 eggs/week

Values are mean (95% CI), n=13, controls, n=14, 7 eggs/week, n=15, 12 eggs/week.
Between-group differences were assessed using ANCOVA adjusted for baseline values, age and sex, with the P-values based on Bonferroni-adjusted post-hoc tests.
Paired t-tests were used to assess within-group changes over time, ‡ P<0.001 vs baseline.

The proportion of participants that were classified as vitamin D insufficient and deficient at baseline and after 12 weeks is shown in Table 4. Although the change (increase) in the proportion of participants classified as vitamin D insufficient and deficient from baseline to 12 weeks was greatest in controls (2 eggs/week), chi-square analysis revealed there was no statistically significant group differences in the proportion of participants changed vitamin D categories (e.g. from insufficient to deficient, from deficient to insufficient, from deficient to adequate, from insufficient to adequate).

Table 4 Number and proportion (%) of participants that were classified as having a vitamin D status classified as insufficient (serum 25(OH)D <75 nmol/L) and deficient (serum 25(OH)D <50 nmol/L) at baseline and after the 12-week intervention

	Vitamin D insufficiency (serum 25(OH)D <75 nmol/L)				**Vitamin D deficiency (serum 25(OH)D <50 nmol/L]**			
Groups	*n*	*Baseline*	*n*	*12 weeks*	*n*	*Baseline*	*n*	*12 weeks*
Control (2 eggs/week)	17	7 (41%)	13	10 (77%)	17	1 (6%)	13	5 (39%)
7 eggs/week	17	9 (53%)	14	10 (71%)	17	4 (24%)	14	5 (36%)
12 eggs/week	17	8 (47%)	15	6 (40%)	17	2 (12%)	15	3 (20%)

3.6 Blood lipids

There were no between-group differences or within-group changes for any blood lipid measure, with the exception that total cholesterol (*P* = 0.017) and LDL-cholesterol (*P* = 0.036) increased significantly after 12 weeks in the 12 eggs/week group (Table 5).

Table 5 Mean baseline values and changes after 12 weeks in weight, serum 25(OH)D and blood lipid concentrations in the control, 7 and 12 eggs per week groups[1]

	Control		7 eggs/week		12 eggs/week		
	Baseline (n=17)	**Change[2] (n=13)**	**Baseline (n=17)**	**Change[2] (n=14)**	**Baseline (n=17)**	**Change[2] (n=15)**	*P-value*[3]
Weight, kg	67.0 ± 12.5	0.3 (-1,0, 1,6)	76.1 ± 25.4	0.0 (-0.9, 0.9)	71.5 ± 15.4	0.7 (-0.1, 1.4)	*0.561*
Serum 25(OH)D, nmol/L	84.4 ± 28.7	-28.6 (-38.1, -18.9) ‡	74.2 ± 28.1	-8.3 (-17.0, 0.4) [a]	79.5 ± 25.1	-7.2 (-18.6, 4.3) [b]	*0.003*
Total cholesterol, mmol/l	4.79 ± 1.21	0.22 (-0.07, 0.52)	4.63 ± 0.86	0.27 (-0.15, 0.70)	4.68 ± 0.99	0.47 (0.09, 0.84) *	*0.440*
HDL-cholesterol, mmol/l	1.56 ± 0.42	-0.01 (-0.08, 0.07)	1.35 ± 0.20	0.07 (-0.07, 0.20)	1.62 ± 0.33	0.05 (-0.07, 0.16)	*0.721*
LDL-cholesterol, mmol/l	2.81 ± 1.19	0.21 (-0.04, 0.46)	2.73 ± 0.65	0.16 (-0.19, 0.52)	2.64 ± 0.98	0.41 (0.05, 0.76) *	*0.444*
Triglycerides, mmol/l	0.95 ± 0.42	0.02 (-0.12, 0.17)	1.21 ± 0.78	0.07 (-0.13, 0.29)	0.91 ± 0.37	0.03 (-0.14, 0.21)	*0.888*

[1] All baseline values are unadjusted means ± standard deviations (SD).

[2] All within-group changes from baseline are unadjusted means [95% confidence interval (CI)] and were assessed by paired t-tests. *P<0.05; ‡ P<0.001 versus baseline. HDL- high density lipoprotein cholesterol; LDL- low density lipoprotein cholesterol.

[3] Interaction P-values for between-group differences were assessed using ANCOVA adjusted for baseline values, age and sex; [a] P<0.05, [b] P<0.01 versus change in controls.

3.7 Sun exposure and protection practices

On average, the vast majority (71–82%) of participants in all three groups reported that they spent on average, 30 minutes or less outdoors on the weekday between 10am and 2pm throughout the entire 12-week study period. While the time spent outdoors was greater on the weekends, 69–82% of all participants reported that they spent 60 minutes or less outdoors on the weekend (Table 6). There were no group differences in sun exposure habits at any time throughout the study, with the exception that participants in the control and 7 eggs/week group tended to spend greater time outdoors on the weekend at week 6 and during the weekdays at week 12 (Table 6). Overall, 86–100% of participants reported that they undertook safe sun protection practices at all times throughout the study.

Table 6 Percentage of participants in the control, 7 and 12 eggs per week group that spent different times outdoors on the weekdays and weekend days between 10am and 2pm at baseline, 3, 6, 9 and 12 weeks[1]

	Weekdays (10am–2pm)				**Weekend days (10am–2pm)**			
	<15 min	*15-30 min*	*30-60 min*	*>60 min*	*<15 min*	*15-30 min*	*30-60 min*	*>60 min*
Baseline								
Controls	30%	35%	35%	0%	12%	29%	47%	12%
7 eggs/week	30%	35%	35%	0	12%	12%	35%	41%
12 eggs/week	47%	35%	6%	12%	6%	35%	18%	41%
Chi-square		*P=0.22*				*P=0.09*		
Week 3								
Controls	40%	27%	33%	0%	13%	27%	47%	13%
7 eggs/week	25%	38%	25%	12%	0%	38%	31%	31%
12 eggs/week	50%	38%	6%	6%	6%	35%	18%	41%
Chi-square		*P=0.38*				*P=0.61*		
Week 6								
Controls	40%	33%	20%	7%	33%	27%	47%	13%
7 eggs/week	40%	33%	20%	7%	20%	13%	27%	40%
12 eggs/week	53%	47%	0%	0%	7%	60%	13%	20%
Chi-square		*P=0.56*				*P=0.05*		
Week 9								
Controls	47%	20%	26%	7%	13%	27%	33%	27%
7 eggs/week	27%	33%	33%	7%	7%	13%	53%	27%
12 eggs/week	50%	43%	7%	0%	7%	50%	21%	22%
Chi-square		*P=0.47*				*P=0.29*		
Week 12								
Controls	36%	43%	0%	21%	21%	21%	21%	37%
7 eggs/week	42%	25%	25%	8%	8%	17%	50%	25%
12 eggs/week	38%	63%	0%	0%	6%	31%	38%	25%
Chi-square		*P=0.04*				*P=0.62*		

[1] All values are percentages.
min – minutes.

3.8 Egg acceptability

Overall, participants rated highly that they liked eating the eggs and their taste, and that it was not too difficult or too much effort to consume their prescribed dose of eggs (Table 7). There were no significant differences between the three groups to any of the egg acceptability questions, but there was a trend for the median scores to the questions *'how well did you like the taste of eggs?'* and '*how easy or difficult was it for you to prepare the eggs to eat?'* to be lower in the 12 eggs/week group (both $P = 0.06$).

3.9 Per protocol analysis

All results (between-group effects) remained unchanged following the per-protocol analysis, which included the 38 participants (n = 12 controls, n = 13 7 eggs/week, n = 13 12 eggs/week) that achieved $\geq$ 80% adherence to eggs (data not shown).

Table 7 Egg consumption acceptability as rated on a food acceptability questionnaire at the end of the 12-week intervention[1]

Acceptability questions	All (n=38)	Control (n=12)	7 eggs/week (n=12)	12 eggs/week (n=14)	*P-value*[2]
How well did you like eating the eggs? *(1=Not at all, 7=Extremely)*	6.0 (5.0, 7.0)	6.5 (6.0, 7.0)	6.0 (5.0, 7.0)	5.0 (3.8, 5.3)	*P=0.225*
How well did you like the taste of the eggs? *(1=Not at all, 7=Extremely)*	6.0 (5.0, 7.0)	7.0 (6.0, 7.0)	6.0 (5.0, 7.0)	5.0 (4.0, 5.3)	*P=0.06*
How easy or difficult was it for you to prepare the eggs to eat? *(1=Extremely difficult, 7=Extremely easy)*	7.0 (5.0, 7.0)	7.0 (7.0, 7.0)	7.0 (6.0, 7.0)	4.5 (3.8, 7.0)	*P=0.06*
How much effort did it take for you to consume the eggs? *(1=A lot of effort, 7=No effort at all)*	5.0 (3.0, 7.0)	7.0 (3.3, 7.0)	5.0 (3.5, 6.0)	5.0 (2.0, 6.0)	*P=0.135*
How satisfied did you feel after eating eggs? *(1=Extremely dissatisfied, 7=Extremely satisfied)*	5.0 (5.0, 6.0)	5.5 (5.0, 7.0)	6.0 (5.0, 6.8)	5.0 (4.0, 6.0)	*P=0.492*
Did eating out influence your ability to consume the eggs as part this study? *(1=Never, 7=Always)*	2.0 (1.0, 5.0)	2.0 (1.0, 5.0)	2.0 (1.0, 4.0)	2.0 (1.0, 5.0)	*P=0.657*
How easy could you to continue consuming the same number of eggs after completion of the study *(1=Extremely difficult, 7=Extremely easy)*	4.0 (3.0, 6.3)	5.0 (3.0, 7.0)	5.0 (4.0, 6.8)	4.0 (2.0, 4.3)	*P=0.155*

[1] All values are median and interquartile range in brackets.

[2] P-values for between-group difference were assessed using Chi-square tests.
One participant from each group did not complete the acceptability questionnaire at follow-up.

4 Discussion

The main finding from this 12-week RCT was that consumption of 7 or 12 commercially available eggs/week was equally effective for attenuating the wintertime decrease in serum 25(OH)D concentrations in young Australian adults. Furthermore, participant acceptability profiles in relation to consuming the eggs were positive with no significant differences between those consuming 2, 7 or 12 eggs/week. Finally, there was no effect (group differences) on the secondary outcomes of body weight, or any blood lipid measures. Collectively, these findings indicate that consumption of 7 commercially available eggs/week, which is in line with the current Australian dietary guidelines (NHMRC 2013), represents a safe and effective dietary approach to attenuate the wintertime decrease in circulating 25(OH)D concentrations in young Australian adults residing in southern Australia.

The finding that consumption of 7 eggs/week, which provided a weekly vitamin D dose of ~36 µg (~1450 IU), was effective for reducing the wintertime decline in serum 25(OH)D concentrations compared to controls (2 eggs/week), is similar to the results from a previous 8-week trial in adults aged 45–70 years in which 7 vitamin D-enriched egg [vitamin D_3 or 25(OH)D_3 eggs] per week [providing 25–32 µg (990–1270 IU) of vitamin D per week] prevented the wintertime decrease in 25(OH)D concentrations (Hayes et al. 2016). However, there are several differences in the findings from these trials. First, in our study mean 25(OH)D concentrations decreased by 8.3 nmol/L (P = 0.061) or 9.7 nmol/L (P = 0.036) after excluding one participant that commenced taking vitamin D supplements, following the consumption of 7 eggs/week for 12 weeks, whereas in the previous 8-week trial serum 25(OH)D concentrations remained unchanged [mean change: vitamin D_3 eggs = 2.2 nmol/L; 25(OH)D_3 eggs = -0.2 nmol/L]. Second, the mean wintertime decrease in serum 25(OH)D after 12 weeks in the controls (2 eggs/week) in our study was 28.6 nmol/L, which was considerably greater than the mean 6.4 nmol/L reduction after 8 weeks reported in the controls (who habitually consumed ≤ 2 eggs/week) in this previous study (Hayes et al. 2016). This is also greater than the mean 12 to 23 nmol/L wintertime reduction we have observed in previous Australian epidemiologic studies (Daly et al. 2012; Malacova et al. 2019) and a 16-week trial in middle-aged women (Daly et al. 2020). These contrasting results may relate to differences in the age range (45–70 vs 25–40 years) and BMI (~25 vs 33) of participants, study duration (8 vs 12 weeks) and geographical location (latitude), all of which are known to influence vitamin D status, and the higher mean baseline serum 25(OH)D concentrations in our study (74–84 vs 41–49 nmol/L). For instance, previous research has shown that the serum 25(OH)D responses to vitamin D supplementation or vitamin D fortified foods are influenced by baseline concentrations, with greater changes observed in those with lower initial 25(OH)D concentrations (Kaykhaei et al. 2019; Nikooyeh & Neyestani 2021; Black et al. 2012). However, this still does not explain why we observed a marked decrease in serum 25(OH)D concentrations in our study given that both trials used the same dose of eggs (n = 7 per week) that provided a similar weekly amount of vitamin D, although it is worth noting that this study was conducted during a lockdown period in Melbourne due to COVID-19.

Other factors known to contribute to differences (changes) in serum 25(OH)D concentrations include habitual dietary vitamin D intake and sun exposure habits. In our study, habitual vitamin D intakes could not be determined as there is no comprehensive food database available (or included in the ASA24 dietary platform) on the vitamin D content of foods in Australia. However, previous research has reported the average dietary vitamin D intakes of Australian adults is approximately 2–4 µg/day (Jayaratne et al. 2013; Nowson et al. 2012). Based on this estimate, the total daily vitamin D intake of participants in the 7 eggs/week group in our study would be 7–9 µg/day, which is comparable to the 9.5–10.4 µg/day (total vitamin intake) reported in the previous 8-week intervention by Hayes and colleagues (Hayes et al. 2016). Thus, it is unlikely that dietary vitamin D intakes contributed to the different findings although it is possible that dietary vitamin D intake may have been underestimated in previous studies given that newer testing methods are available for detecting vitamin D in foods. Since sun exposure is the main source of vitamin D, unique to our study is that we assessed habitual

sun exposure and sun protection practices every 3 weeks throughout the intervention. In Australia, current guidelines recommend 2–3 hours per week of sun exposure (face, arms, hands or equivalent; 3–6 times this amount for dark skin) around midday from May to August in the southern states to meet vitamin D requirements (Ebeling et al. 2013). In our study, 71–82% of participants spent 30 minutes or less per day outdoors on weekdays between 10am and 2pm, which is the peak UV period during May to August, throughout the entire 12-week study period. Although time spent outdoors during this time of the day increased on the weekends, the vast majority (69–82%) of participants spent 60 minutes or less per day outdoors on the weekend. In addition, nearly all (86–100%) participants indicated that they undertook safe sun protection practices at all times throughout the study. Thus, it is possible that this somewhat limited sun exposure and high use of safe sun protection practices contributed to the large decrease in serum 25(OH)D concentrations observed in the controls in our study.

To our knowledge, this is the first RCT to investigate the dose-response effect of eggs on serum 25(OH)D concentrations in younger adults. However, we observed no additional benefit of consuming 12 compared to 7 eggs/week on serum 25(OH)D concentrations, despite the weekly dose of vitamin D increasing to ~62 µg (~2486 IU), assuming a conversion factor of five for 25(OH)D_3. It is difficult to explain these findings since adherence was similar for both groups (mean 83–86%) and previous research has shown that serum 25(OH)D concentrations increase by around 1–2 nmol/L for every additional 100 IU of vitamin D_3 (Cranney et al. 2007), including with the use of vitamin D fortified foods (Nikooyeh & Neyestani 2021; Black et al. 2012). However, as stated above our estimation of the total vitamin D content of the eggs included a conversion factor of five for the 25(OH)D_3 content of the eggs (Hayes et al. 2016). Others have reported lower conversion factors (~1 to 3) (Rossini et al. 2005; Jetter et al. 2014), which if applied to our 7 and 12 eggs/week groups would translate to an additional 16.4 to 28.1 µg (656 to 1124 IU) of vitamin D per week based on a conversion factor of 1 or 26.3 to 45.1 µg (1052 to 1804 IU) of vitamin D per week based on a conversion factor of 3 (National Health and Medical Research Council (NMHRC) 2016). Intakes of vitamin D at these levels (equivalent to 2.3 to 4.0 µg/d and 3.8 to 6.4 µg/d) are mostly below the current AI of vitamin D for Australians aged 1–50 years of 5 µg/d. It has been acknowledged that the current guidelines for the AI of vitamin D in Australia are outdated (Nowson et al. 2012), with intakes of 10–15 µg/d (400–600 IU/d) typically recommended to maintain adequate vitamin D status throughout the year (Holick et al. 2011; Nowson et al. 2012; Ross et al. 2011). Despite the lack of an increase in serum 25(OH)D concentrations in either the 7 or 12 eggs/week group in our study, the finding that there was a significant net benefit of ~20 nmol/L relative to controls (2 eggs/week) is in line with the findings from a previous meta-analysis reporting that vitamin D intakes of ~11 µg/d (~440 IU/d) from fortified foods resulted in a treatment effect of 19.4 nmol/L (Black et al. 2012).

Given that consumption of 12 eggs/week exceeds the recommendation of the Australian Dietary Guidelines of 7 eggs/week (NHMRC 2013), it was important to understand the participants' acceptability to eating the different doses of eggs prescribed in this study. All participants rated highly that they liked eating the eggs and their taste, and that it was not too difficult or too much effort to consume their prescribed dose of eggs. Although there were no significant differences in acceptability scores between the three groups, there was a trend (P = 0.06) for participants in the 12 eggs/week group to report lower scores compared to controls and the 7 eggs/week group to the questions *'how well did you like the taste of eggs?'* and '*how easy or difficult was it for you to prepare the eggs to eat?'*. Acceptability scores for the controls and 7 eggs/week group were almost identical, indicating that any future recommendations to consume 7 egg/weeks to prevent the wintertime decline in 25(OH)D is likely to be regarded as acceptable by young adults. Finally, there were no group differences for the changes in mean body weight or any of the blood lipids concentrations, which is important to alleviate any potential concerns that 7 to 12 eggs/weeks may have adverse health effects. However, it is important to acknowledge that our study was not powered to detect any potential differences for the change blood lipids concentrations, and thus these findings must be interpreted with caution.

Nevertheless, our results are consistent with the findings from several previous trials over 8 to 12 weeks showing that consumption of 7 or 12 eggs week did not have any adverse effects on blood lipids profile when comparing changes between the groups (Fuller et al. 2015; Hayes et al. 2016), but there was an increase in total cholesterol and LDL-cholesterol in the 12 eggs/week group in our study.

The strengths of this study include that it is the first three-arm, randomised controlled trial to evaluate the dose-response effects of consuming eggs on serum 25(OH)D concentrations, the high adherence to the different doses of eggs, the 12-week intervention period since circulating 25(OH)D concentrations tend to plateau around this time after vitamin D treatment (Nowson et al. 2012), and the sampling of different batches of the same commercially available eggs from various locations at two timepoints to evaluatetheir vitamin D content.

Limitations include the lack of assessment of dietary vitamin D intake, the use of a self-report questionnaire to assess habitual sun exposure habits rather than a more accurate measure such as a UV exposure dosimeter, and self-report of body weight, which was necessary due to COVID restrictions. The high baseline serum 25(OH)D concentrations of participants are also a potential limitation given that the response to vitamin D treatment is typically greater in those with lower initial 25(OH)D concentrations (Kaykhaei et al. 2019; Nikooyeh & Neyestani 2021; Black et al. 2012). Therefore, future research should target individuals who are either vitamin D deficient or insufficient.

In conclusion, this study indicates that consumption of 7 commercially available eggs per week, which is in line with the current Australian dietary guidelines (NHMRC 2013), was safe, acceptable, and effective for attenuating the wintertime decrease in serum 25(OH)D concentrations in young Australian adults. Consuming 12 eggs per week did not result in any added benefits to serum 25(OH)D concentrations over 7 eggs per week, but did result in an increase in daily protein intake. These findings indicate that weekly consumption of 7 eggs should be included as an important dietary approach to help to optimise vitamin D status during the winter months in Australia.

5 References

Amrein, K., Scherkl, M., Hoffmann, M., Neuwersch-Sommeregger, S., Kostenberger, M., Tmava Berisha, A., Martucci, G., Pilz, S. & Malle, O. 2020. Vitamin D deficiency 2.0: an update on the current status worldwide. *Eur J Clin Nutr,* 74**,** 1498-1513.

Barnard, N. D., Gloede, L., Cohen, J., Jenkins, D. J., Turner-Mcgrievy, G., Green, A. A. & Ferdowsian, H. 2009. A low-fat vegan diet elicits greater macronutrient changes, but is comparable in adherence and acceptability, compared with a more conventional diabetes diet among individuals with type 2 diabetes. *J Am Diet Assoc,* 109**,** 263-72.

Black, L. J., Seamans, K. M., Cashman, K. D. & Kiely, M. 2012. An updated systematic review and meta-analysis of the efficacy of vitamin D food fortification. *J Nutr,* 142**,** 1102-8.

Black, L. J., Jacoby, P., Nowson, C. A., Daly, R. M. & Lucas, R. M. 2016. Predictors of Vitamin D-Containing Supplement Use in the Australian Population and Associations between Dose and Serum 25- Hydroxyvitamin D Concentrations. *Nutrients,* 8.

Brown, W. J., Burton, N. W., Marshall, A. L. & Miller, Y. D. 2008. Reliability and validity of a modified self-administered version of the Active Australia physical activity survey in a sample of mid-age women. *Aust N Z J Public Health,* 32**,** 535-41.

Cashman, K. D., Seamans, K. M., Lucey, A. J., Stocklin, E., Weber, P., Kiely, M. & Hill, T. R. 2012. Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. *Am J Clin Nutr,* 95**,** 1350-6.

Cranney, A., Horsley, T., O'donnell, S., Weiler, H., Puil, L., Ooi, D., Atkinson, S., Ward, L., Moher, D., Hanley, D., Fang, M., Yazdi, F., Garritty, C., Sampson, M., Barrowman, N., Tsertsvadze, A. & Mamaladze, V. 2007. Effectiveness and safety of vitamin D in relation to bone health. *Evid Rep Technol Assess (FullRep)***,** 1-235.

Daly, R. M., Gagnon, C., Lu, Z. X., Magliano, D. J., Dunstan, D. W., Sikaris, K. A., Zimmet, P. Z., Ebeling, P. R. & Shaw, J. E. 2012. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. *Clin Endocrinol (Oxf),* 77**,** 26-35.

Daly, R. M., Gianoudis, J., De Ross, B., O'connell, S. L., Kruger, M., Schollum, L. & Gunn, C. 2020. Effects of a multinutrient-fortified milk drink combined with exercise on functional performance, muscle strength, body composition, inflammation, and oxidative stress in middle-aged women: a 4-month, double-blind, placebo-controlled, randomized trial. *Am J Clin Nutr,* 112**,** 427-446.

De Leenheer, A. P., Lambert, W. E., De Ruyter, M. G. M. & (Eds) 1985. *Modern Chromatographic Analysis of the Vitamins,* New York, NY, Marcel Dekker Inc.

Dunlop, E., Cunningham, J., Sherriff, J. L., Lucas, R. M., Greenfield, H., Arcot, J., Strobel, N. & Black, L. J. 2017. Vitamin D(3) and 25-Hydroxyvitamin D(3) Content of Retail White Fish and Eggs in Australia. *Nutrients,* 9.

Dunlop, E., James, A. P., Cunningham, J., Strobel, N., Lucas, R. M., Kiely, M., Nowson, C. A., Rangan, A.,Adorno, P., Atyeo, P. & Black, L. J. 2021. Vitamin D composition of Australian foods. *Food Chem,* 358**,** 129836.

Ebeling, P. R., Daly, R. M., Kerr, D. A. & Kimlin, M. G. 2013. Building healthy bones throughout life: an evidence-informed strategy to prevent osteoporosis in Australia. *Med J Aust,* 199**,** S1-S46.

Fitzpatrick, T. B. 1988. The validity and practicality of sun-reactive skin types I through VI. *Arch Dermatol,* 124**,** 869-71.

Fuller, N. R., Caterson, I. D., Sainsbury, A., Denyer, G., Fong, M., Gerofi, J., Baqleh, K., Williams, K. H., Lau, N. S. & Markovic, T. P. 2015. The effect of a high-egg diet on cardiovascular risk factors in people with type 2 diabetes: the Diabetes and Egg (DIABEGG) study-a 3-mo randomized controlled trial. *Am JClin Nutr,* 101**,** 705-13.

Hayes, A., Duffy, S., O'grady, M., Jakobsen, J., Galvin, K., Teahan-Dillon, J., Kerry, J., Kelly, A., O'doherty, J., Higgins, S., Seamans, K. M. & Cashman, K. D. 2016. Vitamin D-enhanced eggs are protective of wintertime serum 25-hydroxyvitamin D in a randomized controlled trial of adults. *Am J Clin Nutr,* 104**,** 629-37.

Heesch, K. C., Hill, R. L., Van Uffelen, J. G. & Brown, W. J. 2011. Are Active Australia physical activity questions valid for older adults? *J Sci Med Sport,* 14**,** 233-7.

Hilger, J., Friedel, A., Herr, R., Rausch, T., Roos, F., Wahl, D. A., Pierroz, D. D., Weber, P. & Hoffmann, K. 2014. A systematic review of vitamin D status in populations worldwide. *Br J Nutr,* 111**,** 23-45.

Holick, M. F., Binkley, N. C., Bischoff-Ferrari, H. A., Gordon, C. M., Hanley, D. A., Heaney, R. P., Murad, M. H., Weaver, C. M. & Endocrine, S. 2011. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab,* 96**,** 1911-30.

Jayaratne, N., Hughes, M. C., Ibiebele, T. I., Van Den Akker, S. & Van Der Pols, J. C. 2013. Vitamin D intake in Australian adults and the modeled effects of milk and breakfast cereal fortification. *Nutrition,* 29**,** 1048-53.

Jetter, A., Egli, A., Dawson-Hughes, B., Staehelin, H. B., Stoecklin, E., Goessl, R., Henschkowski, J. & Bischoff-Ferrari, H. A. 2014. Pharmacokinetics of oral vitamin D(3) and calcifediol. *Bone,* 59**,** 14-9.

Kaykhaei, M. A., Khodadoost, M., Dashipour, A. R., Haidari, Z., Karimkoshteh, A. & Sandoughi, M. 2019. Baseline levels determine magnitude of increment in 25 hydroxy vitamin D following vitamin D3 prescription in healthy subjects. *Endocrine,* 64**,** 378-383.

Malacova, E., Cheang, P. R., Dunlop, E., Sherriff, J. L., Lucas, R. M., Daly, R. M., Nowson, C. A. & Black, L. J. 2019. Prevalence and predictors of vitamin D deficiency in a nationally representative sample of adults participating in the 2011-2013 Australian Health Survey. *Br J Nutr,* 121**,** 894-904.

National Health and Medical Research Council (NHMRC) 2013. Australian Dietary Guidelines. Canberra: National Health and Medical Research Council.

National Health and Medical Research Council (NHMRC) 2016. Nutrient reference values for Australia and New Zealand: Vitamin D.

Nikooyeh, B. & Neyestani, T. R. 2021. The effects of vitamin D-fortified foods on circulating 25(OH)D concentrations in adults: a systematic review and meta-analysis. *Br J Nutr***,** 1-18.

Nowson, C. A., Mcgrath, J. J., Ebeling, P. R., Haikerwal, A., Daly, R. M., Sanders, K. M., Seibel, M. J., Mason, R. S., Working Group Of, A., New Zealand, B., Mineral Society, E. S. O. A. & Osteoporosis, A. 2012. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust,* 196**,** 686-7.

Ovesen, L., Brot, C. & Jakobsen, J. 2003. Food contents and biological activity of 25-hydroxyvitamin D: a vitamin D metabolite to be reckoned with? *Ann Nutr Metab,* 47**,** 107-13.

Ross, A. C., Manson, J. E., Abrams, S. A., Aloia, J. F., Brannon, P. M., Clinton, S. K., Durazo-Arvizu, R. A.,Gallagher, J. C., Gallo, R. L., Jones, G., Kovacs, C. S., Mayne, S. T., Rosen, C. J. & Shapses, S. A. 2011. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab,* 96**,** 53-8.

Rossini, M., Viapiana, O., Gatti, D., James, G., Girardello, S. & Adami, S. 2005. The long term correction of vitamin D deficiency: comparison between different treatments with vitamin D in clinical practice. *Minerva Med,* 96**,** 1-7.