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Serum 25-hydroxyvitamin D response to vitamin D supplementation using different lipid delivery systems in middle-aged and older adults: a randomised controlled trial.

Aislinn F. McCourt[1], Steven L. Mulrooney[1], Graham J. O'Neill[2], E. Dolores O'Riordan[1] and Aifric M. O'Sullivan[1]*

[1]*UCD Institute of Food and Health, University College Dublin (UCD), Dublin, Republic of Ireland*
[2]*TUD School of Food Science and Environmental Health, Technological University Dublin (TUD), Dublin, Republic of Ireland*

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Abstract

Food fortification improves vitamin D intakes but is not yet mandated in many countries. Combining vitamin D with different dietary lipids altered vitamin D absorption in *in vitro* and postprandial studies. This randomised, placebo-controlled trial examined the effect of the lipid composition of a vitamin D-fortified dairy drink on change in 25-hydroxyvitamin D (25(OH)D) concentrations. Sixty-three healthy adults aged 50+ years were randomised to one of the following for 4 weeks: vitamin D-fortified olive oil dairy drink, vitamin D-fortified coconut oil dairy drink, vitamin D supplement or placebo control dairy drink. All vitamin D groups received 20 µg of vitamin D_3 daily. Serum was collected at baseline and post-intervention to measure 25(OH)D concentrations and biomarkers of metabolic health. Repeated-measures general linear model ANCOVA (RM GLM ANCOVA) compared changes over time. There was a significant time × treatment interaction effect on 25(OH)D concentrations for those classified as vitamin D-insufficient ($P < 0{\cdot}001$) and -sufficient at baseline ($P = 0{\cdot}004$). 25(OH)D concentrations increased significantly for all insufficient participants receiving vitamin D_3 in any form. However, for vitamin D-sufficient participants at baseline, 25(OH)D concentrations only increased significantly with the coconut oil dairy drink and supplement. There was no effect of vitamin D on biomarkers of metabolic health. Vitamin D fortification of lipid-containing foods may be used in lieu of supplementation when supplement adherence is low or for individuals with dysphagia. These results are important given the recent recommendation to increase vitamin D intakes to 15–20 µg for older adults in Ireland.

Key words: **Vitamin D: Food fortification: Older adults: Vitamin D deficient: 25-hydroxyvitamin D**

Vitamin D is a fat-soluble vitamin necessary for Ca homoeostasis, immune function and insulin sensitivity[1]. Research evidence shows inverse associations between 25-hydroxyvitamin D (25(OH)D) concentrations inflammation, serum lipid concentrations and markers of glucose metabolism[1]. Vitamin D status is measured by circulating 25(OH)D concentrations, with the Institute of Medicine (IOM) defining deficiency as 25(OH)D < 30 nmol/l and suggesting 50 nmol/l as the serum level that meets the needs of most people[2]. For the purpose of this research, the authors define concentrations of ≥ 50 nmol/l, < 50 nmol/l and < 30 nmol/l as sufficient, insufficient and deficient, respectively; with ≥ 125 nmol/l, the concentration at which risk of adverse effects is increased[2]. Vitamin D sources include food, dietary supplements and UVB-induced cutaneous synthesis. Insufficiency rates are high in countries with low sun exposure, particularly in older adults due to less time outdoors and lower cutaneous synthesis[3]. This is reflected in national survey data, where 40·1 % of older adults in Ireland are vitamin D-insufficient in winter, compared with 28·9 % in the USA which has a lower latitude[4,5]. High insufficiency rates persist in countries at northerly latitudes despite public health campaigns to increase intakes as a limited number of foods have high concentrations of vitamin D. However, evidence suggests that food fortification increases vitamin D intakes and hence 25(OH)D concentrations, and it also does not require dietary change[6]. A 2012 systematic review and meta-analysis reported an increase of 1·2 nmol/l per 1 µg of fortified vitamin D consumed[7]. Cow's milk is often targeted for fortification as studies report high compliance and no adverse effects[8]; for example, vitamin D-fortified (5 µg) skimmed milk increased 25(OH)D by 8·9 nmol/l over 16 weeks[8]. Similarly, 15 µg of fortified milk increased 25(OH)D by 7·6 nmol/l over 1 year[9]. Consequently, several

Abbreviations: FA, fatty acid; GLM, general linear model; RM, repeated-measures; 25(OH)D, 25-hydroxyvitamin D.

* **Corresponding author:** Aifric M. O'Sullivan, email aifric.osullivan@ucd.ie

countries include cow's milk in national fortification policies, most notably in Finland where mandatory vitamin D fortification increased mean population 25(OH)D concentrations by 18 nmol/l[10–12]. Despite the evidence supporting vitamin D food fortification, many countries have yet to implement a mandatory fortification policy, resulting in low vitamin D intakes and status.

Consuming vitamin D with lipids improves absorption in postprandial studies[13,14]. Using radiolabelled vitamin D, Barragry *et al.* investigated the optimal timing of lipid ingestion following vitamin D consumption[13]. When a lipid-containing meal was consumed with vitamin D, there was a gradual increase in plasma radioactivity over time[13]. However, when vitamin D was consumed alone, plasma radioactivity was not detected until a lipid-containing meal was consumed[13]. More recently, the increased focus on vitamin D deficiency has led to a renewed interest in vitamin D absorption, with new research focusing on the lipid composition of fortified foods[15,16]. For example, in a number of *in vitro* studies, medium-chain fatty acids (FA) with a chain length of 16–18 carbons and MUFA increased vitamin D absorption, compared with longer-chain FA with a chain length of 20 or more carbons and PUFA[15–17]. This is likely due to the medium-chain FA and MUFA forming intestinal micelles that are a favourable size for hosting vitamin D with increased solubility[16,17]. Our recent research has examined this effect *in vivo*[18]. Participants consumed vitamin D-fortified dairy drinks with different oils used to make up the lipid component of the drinks. An olive oil dairy drink was more effective at increasing postprandial 25(OH)D concentrations compared with a non-lipid or fish oil dairy drink in vitamin D-insufficient participants (unpublished results, McCourt *et al.*). However, no studies have examined the effect of different dietary oils on 25(OH)D concentrations over time.

Food fortification policies increase 25(OH)D concentrations at a population level, yet are not widely implemented. As so little is known about vitamin D absorption, we should ensure that vitamin D can be absorbed from fortified foods before implementing a fortification policy. Additionally, *in vitro* studies suggest that manipulating the lipid composition of foods may increase vitamin D absorption and thus may increase fortification policy effectiveness. Therefore, this randomised, placebo-controlled trial (RCT) examined changes in 25(OH)D concentrations following daily consumption of a 20 μg of vitamin D_3-fortified dairy drink with olive oil as the lipid component, a 20 μg of vitamin D_3-fortified dairy drink with coconut oil as the lipid component and a 20 μg of vitamin D_3 supplement compared with a placebo control dairy drink in middle-aged and older adults over a 4-week period.

Materials and methods

Outcomes

The primary outcome was change in 25(OH)D concentrations from baseline to post-intervention in each of the vitamin D groups compared with the placebo control dairy drink group. Given the reported associations with serum lipids, inflammation and makers of glucose metabolism, changes in biomarkers of metabolic health (e.g. cholesterol and glucose) from baseline to post-intervention were compared as secondary outcomes. Factors contributing to response to vitamin D intervention (significant increase in 25(OH)D) were also examined.

Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human participants were approved by the University College Dublin (UCD) Human Research Ethics Committee (LS-19-69-McCourt-O'Sullivan). Written informed consent was obtained from all participants. The study was registered at clinicaltrials.gov (NCT04156074).

Participants

Healthy adults aged 50+ years were recruited in Dublin, Ireland, 53·3°N between October 2019 and March 2020. The trial was stopped slightly ahead of schedule in March 2020 due to COVID-19 restrictions. Exclusion criteria included < 50 years; smokers[19], institutionalised individuals, an inability to read, write or understand English, any prescribed or weight loss diet, any disease or condition that requires medical or nutritional treatment, consuming supplemental vitamin D or, sun holiday in the last 2 months or during the study, and milk protein allergy and lactose intolerance.

Study design

This was a RCT with four study groups including three dairy drinks and one supplement. Volunteers who met the inclusion criteria signed informed consent and were randomised in a 1:1:1:1 ratio using a computer-based number generator. No restrictions were applied to the randomisation protocol. Participants and the researcher conducting study visits were blinded to the composition of the dairy drinks which were identical in appearance and taste. The researcher who made the dairy drinks was not involved in the study visits or data collection. It was not possible to blind the vitamin D supplement group. The three drinks were a vitamin D-fortified dairy drink with olive oil or coconut oil as the lipid component, or a placebo control dairy drink with coconut oil as the lipid component. Olive oil and coconut oil were the chosen lipid components to compare the potential effects of the FA chain length and degree of saturation on vitamin D absorption. The drinks were composed of 1·5 % oil, 6·0 % milk protein isolate, 5·0 % lactose and 87·5 % water to reflect the macro-nutrient composition of low-fat milk (Table 1). Each vitamin D group contained 20 μg of vitamin D_3 as this previously increased 25(OH)D concentrations above 50 nmol/l in older adults in Ireland[20,21]. Participants were asked to consume a 30 ml of dairy drink or two vitamin D supplements daily upon waking, before consuming any other food for 4 weeks. A 4-week intervention period was chosen as evidence from the literature shows that vitamin D supplementation at levels similar to the current study elicited a significant increase in 25(OH)D at 4 weeks[22,23]. Data and samples were collected at baseline (visit 1) and post-intervention (visit 3). The participants also came to the study centre at the mid-point (visit 2) to collect additional drinks/supplements and to return their food diary. Participants kept the

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Table 1. Nutritional composition of the dairy drinks per 30 ml serving

	Energy (MJ)	Fat (g)	Carbohydrate (g)	Protein (g)	Vitamin D_3 (μg)
Olive oil drink	0·07	0·45	1·5	1·8	20
Coconut oil drink	0·07	0·45	1·5	1·8	20
Placebo drink	0·07	0·45	1·5	1·8	0

drinks bottles or supplement packs, and compliance was calculated as the number of days that the drink or supplement was consumed, divided by the number of days between visit 1 and visit 3 to yield a percentage.

Anthropometric measurements

Fasted anthropometric measurements were recorded using standardised protocols. Height was measured to the nearest 0·5 cm (freestanding SECA stadiometer, Leicester MkII), weight was measured to the nearest 0·01 kg (Tanita scale, Model BC-420MA) and waist circumference was measured to the nearest 0·1 cm (non-stretch tape). Body composition was measured (Tanita scale, Model BC-420MA) and BMI calculated (weight (kg)/height $(m)^2$).

Diet and lifestyle assessment

Habitual food and drink intake was assessed using a 4-d food diary. Participants were asked to record the amount, type and brand of all food, drinks, supplements and medications consumed during four consecutive days, including at least 1 weekend day, in the first 2 weeks. Food weights were recorded where possible or estimated using standard portion sizes and household measures. Dietary intake was entered into Nutritics software (Nutritics Research Edition, v5.095) for analysis. Data were quality controlled for accuracy by rechecking the foods and weights entered for all food diaries. Nutrient intake data were exported to IBM SPSS Statistics, version 24 (IBM Corp.), and mean daily nutrient intakes were calculated. Underreporters of energy intake were identified as having a ratio of energy intake to basal metabolic rate of < 1·1, which was calculated using the Henry equation[24]. All analyses were run for the full cohort and excluding underreporters (n 7). There was no difference in the results from both; therefore, results are presented with underreporters included. Participants completed a health, lifestyle and socio-demographic questionnaire at visit 2 that included questions on employment and marital status, education, overall health perception and physical activity.

Blood sample collection and analysis

Fasted blood samples were collected by a trained phlebotomist into 10 ml clot activator serum tubes (BD Vacutainer). Each sample was inverted five times and clotted for 30 min at room temperature. Samples were centrifuged at 1500RCF for 15 min at 20° C (Rotina 38R). After centrifugation, the samples were aliquoted and stored at −80°C until analysis.

Serum 25(OH)D measurement

25(OH)D was measured as a vitamin D biomarker. 25(OH)D concentrations were assessed by quantification of total 25(OH)D (D_2 and D_3) by a validated method (Chromsystems Instruments and Chemicals GmbH) using LC-tandem MS (LC-MS/MS) (API 4000; AB SCIEX, UK) and analysed in the Biochemistry Department of St James's Hospital (accredited to ISO 15 189)[25,26]. The quality and accuracy of the method was monitored by the use of internal quality controls, participation in the Vitamin D External Quality Assessment Scheme (DEQAS) and the use of the National Institute of Standards and Technology (NIST) 972 vitamin D standard reference material. The respective inter- and intra-assay CV were 5·7 % and 4·5 %, respectively[25,27]. Vitamin D insufficiency and sufficiency were defined as 25(OH)D concentrations < 50 nmol/l and ≥ 50 nmol/l, respectively. Serum 25(OH)D ≥ 125 nmol/l was considered the concentration at which the risk of adverse effects increased[2].

Biomarkers of metabolic health

Standard commercial kits measured biomarkers of metabolic health according to manufacturer's instructions using the Randox Daytona (Randox Laboratories) and included glucose, total cholesterol (TC), HDL-cholesterol, TAG and C-reactive protein. LDL-cholesterol was calculated using the Friedewald formula[28]: LDL-C = (TC − HDL-C) − (TG/2·17) mmol/l.

Statistical analysis

Sample size was calculated based on previous vitamin D supplementation trials in older adults in Ireland and was powered at 80 % with a type 1 error rate of 0·05[21]. A sample size of 9 in each group is sufficient to detect a difference in 25(OH)D between the vitamin D groups and the placebo control dairy drink group. Statistical analysis was performed in IBM SPSS Statistics, version 24 (IBM Corp.). The distribution of all variables was checked, and boxplots were created to examine outliers. Any variables that were not normally distributed were $\log_{10}$-transformed. Data are presented as mean ± standard error of the mean (se) or median and interquartile range. General linear model ANCOVA (GLM ANCOVA) compared differences in baseline anthropometric, dietary data, 25(OH)D and biomarkers of metabolic health between intervention groups, controlling for appropriate covariates. Sex, age, BMI, body fat percentage, baseline 25(OH)D concentration and vitamin D, fat and Ca intakes were considered as potential covariates for all analyses. GLM ANCOVA was run multiple times for each dependent variable, each time with a different single covariate. Any significant covariates ($P < 0·05$) were included in the final model for the respective dependent variables. Dietary data were also controlled for mean daily energy intake (MJ). Repeated-measures (RM) GLM ANCOVA was used to compare changes in 25(OH)D concentrations and biomarkers of metabolic health from baseline to post-intervention (time × treatment, time and treatment effects). When there was a significant time × treatment interaction, simple main effects of time and treatment were investigated using post hoc pairwise comparisons and GLM ANCOVA. Linear regression

determined predictors of 25(OH)D response in high vitamin D responders. High responders were defined as participants receiving vitamin D with a change in 25(OH)D of ≥ 10 nmol/l from baseline to post-intervention. The enter method was run first, including a range of covariates. Any covariates that were significant or approaching significant ($P < 0{\cdot}07$) were included in a stepwise selection linear regression model.

Results

Participant characteristics

Seventy two participants were randomised and received the allocated intervention (Fig. 1). Nine participants were lost to follow-up for personal reasons or due to COVID-19 restrictions. A total of thirty-two males and thirty-one females aged 60 ± 7 years completed the study (Table 2). All participants self-reported as White, 78 % were educated to tertiary level, 56 % were employed, 8 % were semi-retired and 27 % were retired. Participants had a mean BMI of 28·1 ± 0·9 kg/m^2 at baseline, and there were no differences in anthropometrics or biomarkers of metabolic health between the intervention groups. Based on a 4-d food diary, mean daily vitamin D intake from food was 4·7 ± 0·5 µg.

Baseline vitamin D status

Mean baseline 25(OH)D concentration was 60·3 ± 2·6 nmol/l (Table 3). At baseline, 30 % of participants were vitamin D-insufficient (25(OH)D concentrations < 50 nmol/l) and 70 % were vitamin D-sufficient (25(OH)D concentrations ≥ 50 nmol/l). Thirty-three participants completed the study between November and December (autumn) and 39 between February and March (winter). There was no difference in baseline 25(OH)D concentrations between those who started the study autumn and winter ($P = 0{\cdot}22$).

25(OH)D changes in response to intervention

Fifteen participants completed the olive and coconut oil dairy drink groups, seventeen completed the supplement group and sixteen completed the placebo control dairy drink group (Fig. 1). RM GLM ANCOVA comparing baseline and post-intervention 25(OH)D concentrations and GLM ANCOVA comparing change in 25(OH)D concentration showed significant differences between groups (Table 3). Change in 25(OH)D was significantly different between participants classified as vitamin D-insufficient (< 50 nmol/l) and -sufficient (≥ 50 nmol/l) at baseline; therefore, baseline vitamin D status was included as a between-subject factor when comparing changes in 25(OH)D concentrations. Starting with the insufficient group, two-way RM GLM ANCOVA revealed a significant time × treatment interaction effect on 25(OH)D concentrations ($F(3,14) = 20{\cdot}35$, $P < 0{\cdot}001$, $\eta_p^2 = 0{\cdot}813$) (Table 3). There was also an effect of time on 25(OH)D ($F(1,14) = 15{\cdot}93$, $P = 0{\cdot}001$, $\eta_p^2 = 0{\cdot}532$), and subsequent post hoc analysis revealed a significant 25(OH)D increase over time in the vitamin D groups and a significant decrease in the placebo control dairy drink group. Lastly, there was no effect of treatment on 25(OH)D ($P = 0{\cdot}30$); however, subsequent post hoc analysis revealed higher 25(OH)D in the vitamin D groups compared with the placebo control dairy drink group post-intervention. In the sufficient group, two-way RM GLM ANCOVA highlighted a significant time × treatment interaction effect on 25(OH)D concentrations ($F(3,38) = 5{\cdot}36$, $P = 0{\cdot}005$, $\eta_p^2 = 0{\cdot}297$) (Table 3). There was no main effect of time on 25(OH)D ($P = 0{\cdot}46$); however, subsequent post hoc analysis revealed a significant 25(OH)D increase over time in the coconut oil dairy drink and supplement groups, and no significant change over time in the olive oil dairy drink and placebo control dairy drink groups. Lastly, there was no effect of treatment on 25(OH)D ($P = 0{\cdot}50$), and subsequent post hoc analysis revealed no difference in 25(OH)D between groups post-intervention.

Predictors of 25(OH)D response

Linear regression analysis determined predictors of 25(OH)D response in participants who received 20 µg of vitamin D_3 daily. Independent variables included sex, age, BMI, dietary fat intake, MUFA:PUFA, Ca and vitamin D intakes and baseline 25(OH)D concentrations. Baseline 25(OH)D concentration, sex and PUFA intake were included in the stepwise regression. However, the final model included only baseline 25(OH)D as a significant predictor of response, predicting approximately 69 % of variance in post-intervention 25(OH)D concentrations (adjusted $R^2 = 0{\cdot}685$, $P < 0{\cdot}001$).

Effectiveness of 20 µg of vitamin D at correcting vitamin D insufficiency

Nineteen participants were vitamin D-insufficient at baseline, including five in the olive oil dairy drink group, three in the coconut oil dairy drink group, six in the vitamin D supplement group and five in the placebo control dairy drink group. Only one participant remained vitamin D-insufficient in each of the vitamin D groups post-intervention. However, the 25(OH)D of each of these vitamin D-insufficient participants increased significantly to > 45 nmol/l. The five insufficient participants in the placebo control dairy drink group remained insufficient post-intervention. The vitamin D groups were safe as no participants were at risk of vitamin D-related adverse effects (25(OH)D ≥ 125 nmol/l) and no adverse effects were reported.

Compliance

Compliance was high across the four groups, with a median compliance of 28(26–29) d or 100·0(96·0–100)%. There was no difference in compliance between intervention groups. One participant in the olive oil dairy drink, four in the coconut oil dairy drink and four in the placebo control dairy drink groups missed more than 2 d of intervention.

Changes in biomarkers of metabolic health in response to intervention. There was no significant time × treatment, time or treatment effects on any biomarkers of metabolic health (Table 4). However, there was an increase of 0·26 mmol/l in TC in those with BMI < 27 kg/m^2 from baseline to post-intervention ($P = 0{\cdot}03$). Lastly, those with BMI > 27 kg/m^2 had higher

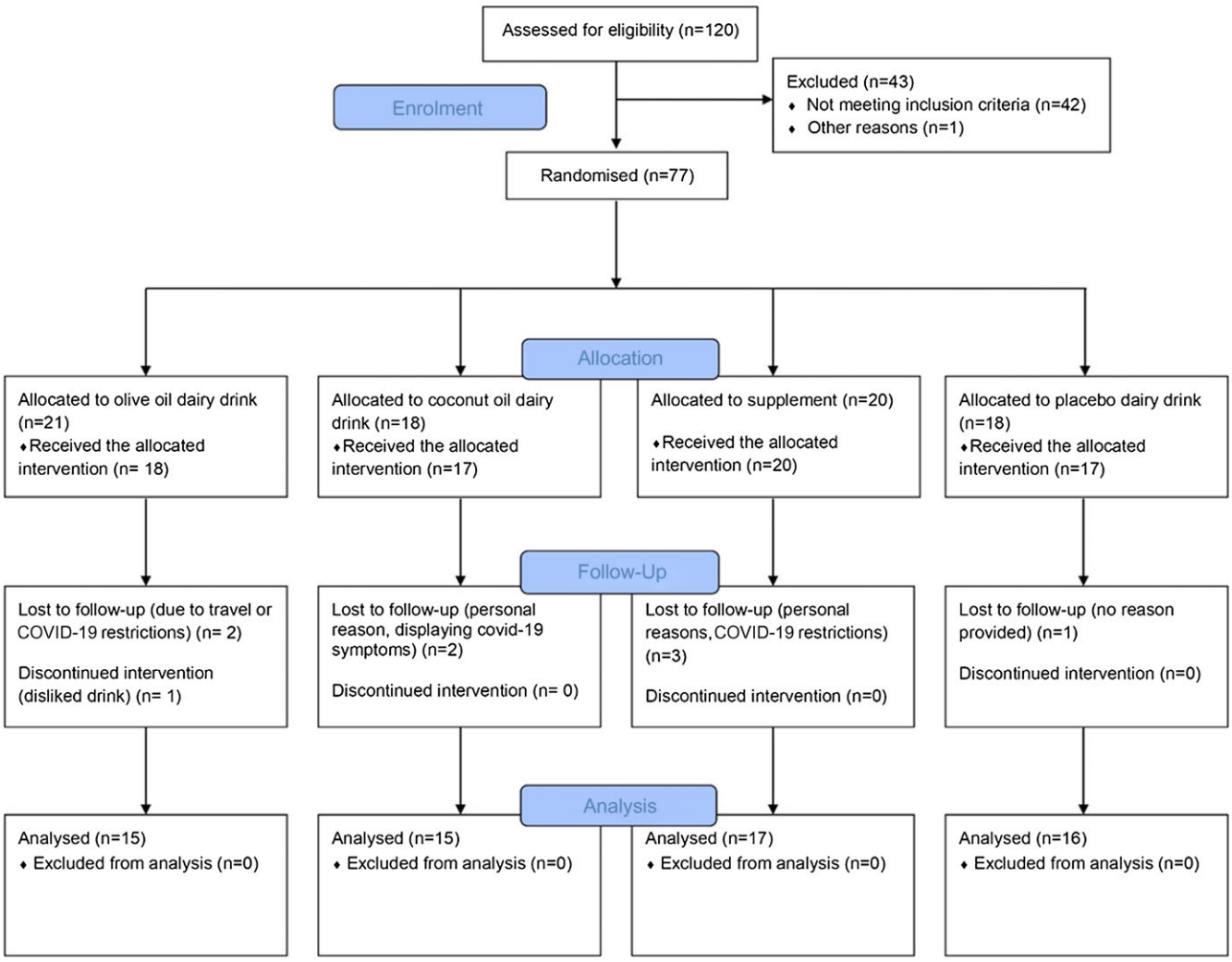

Fig. 1. Flow chart of study progression.

mean TAG ($P < 0{\cdot}001$) and C-reactive protein ($P < 0{\cdot}001$) concentrations at baseline and post-intervention compared with those with BMI < 27 kg/m^2.

Discussion

A 20 µg of vitamin D-fortified dairy drink increased 25(OH)D in middle-aged and older adults. Vitamin D intervention was more effective in vitamin D-insufficient participants than in vitamin D-sufficient participants. 25(OH)D concentrations increased significantly in all vitamin D groups for participants who were vitamin D-insufficient at baseline; whereas the increase in 25(OH)D concentrations for those who were vitamin D-sufficient at baseline were only statistically significantly different compared with the placebo group after the vitamin D-fortified coconut oil dairy drink and the vitamin D supplement. As expected, the placebo control dairy drink did not increase 25(OH)D over time. Baseline 25(OH)D explained approximately 69 % of 25(OH)D response to vitamin D intervention. The majority of participants receiving vitamin D reached sufficiency by the end of the study. Lastly, the interventions did not alter biomarkers of metabolic health. Overall, 20 µg of vitamin D per d was effective at bringing middle-aged and older adults to vitamin D sufficiency over a 4-week period.

To the best of our knowledge, this is the first RCT examining the impact of different dietary lipids in fortified dairy drinks on changes in 25(OH)D. A 20 µg of vitamin D dose is effective at bringing middle-aged and older adults to sufficiency in winter, and dairy drinks are an effective vitamin D delivery vehicle. All vitamin D groups equally raised 25(OH)D in vitamin D-insufficient participants; however, only the coconut oil dairy drink and supplement were effective in sufficient participants. Thus, baseline 25(OH)D may override any effect of FA on vitamin D absorption. We hypothesised that olive oil (about 55–83 % oleic acid (C18:1)) has optimal FA chain length and degree of saturation to increase vitamin D absorption[15,16]. Goncalves *et al.* report that oleic acid and olive oil increase vitamin D efflux from Caco-2 cells compared with other FA and dietary oils[15]. Ozturk *et al.* later reported increased vitamin D bioaccessability from corn oil and fish oil (C16–18) compared with a shorter-chain MCT oil[16]. Following these *in vitro* studies, we previously

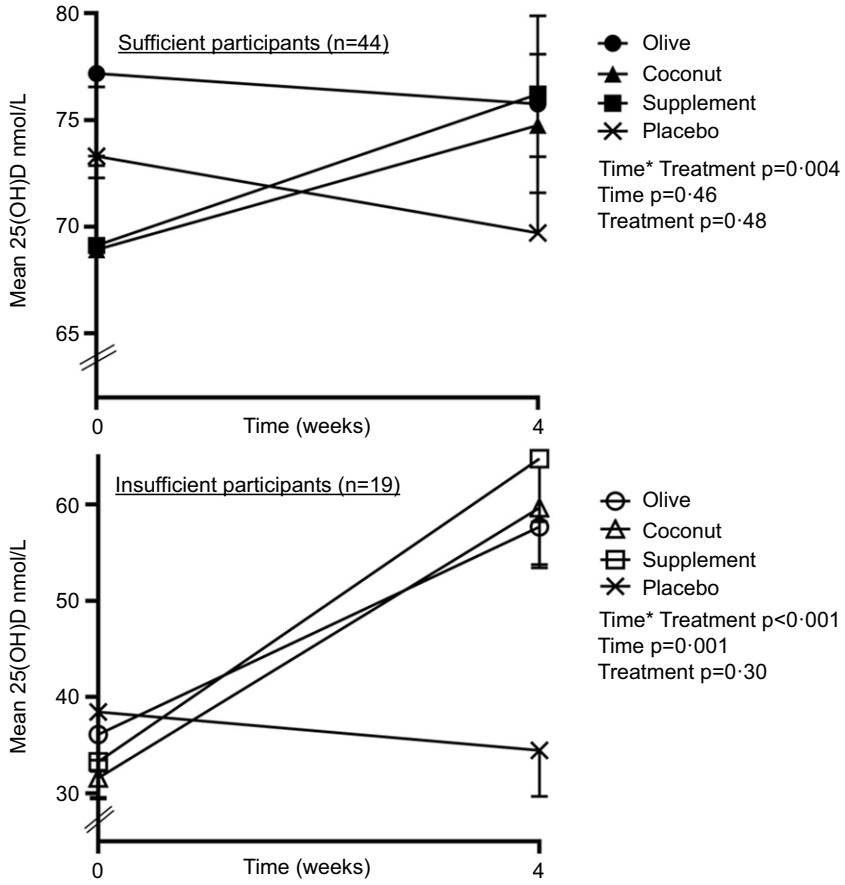

Fig. 2. 25(OH)D response to intervention in vitamin D-sufficient and -insufficient participants using RM GLM ANCOVA.

Table 2. Baseline subject characteristics, including anthropometrics and dietary intake

	Olive		Coconut		Supplement		Placebo		
	n 15		*n* 15		*n* 17		*n* 16		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	*P*
Age (years)	61·0	1·0	62·0	1·0	58·0	1·0	59·0	1·0	0·42
Weight (kg)	79·2	1·9	78·3	1·9	80·6	1·8	82·5	1·9	0·44
Body fat (%)	31·2	0·9	30·3	1·0	32·3	0·9	32·8	1·0	0·25
Protein (%)	16·9	0·7	17·0	0·7	18·5	0·7	17·2	0·7	0·34
Carbohydrate (%)	41·9	1·3	45·8	1·3	44·9	1·3	42·1	1·4	0·10
Fat (%)	37·0	1·1	35·0	1·3	33·5	1·1	38·3	1·1	0·02
Energy (MJ)	9·4	0·5	8·6	0·5	8·1	0·5	8·5	0·5	0·32
Sugars (g)	90·0	8·0	102·4	8·0	84·8	7·8	86·2	8·1	0·40
Saturated fat (g)	32·6	2·3	29·3	2·3	25·4	2·3	32·2	2·4	0·12
MUFA (g)	34·8	2·6	32·9	2·6	27·7	2·6	31·6	2·6	0·26
PUFA (g)	15·8	1·5	15·6	1·5	12·2	1·5	13·6	1·5	0·26
Fibre (g)	23·6	1·7	22·4	1·7	22·0	1·7	21·0	1·7	0·76
Ca (mg)	1127·4	83·5	981·8	83·5	847·5	81·7	845·7	84·1	0·06
Vitamin D (μg)	4·4	0·9	4·9	0·9	4·6	1·0	4·8	1·0	0·98

GLM, general linear model.
Data are presented as mean ± standard error.
GLM ANCOVA, controlled for sex and BMI at baseline, was used to explore differences between groups.
$P < 0·05$ was considered statistically significant.
Olive, vitamin D-fortified olive oil dairy drink; coconut, vitamin D-fortified coconut oil dairy drink; supplement, vitamin D supplement; placebo, placebo control dairy drink.

investigated the effect of an olive oil dairy drink compared with a fish oil dairy drink on postprandial 25(OH)D[18]. However, the olive oil dairy drink was only effective in insufficient participants. Although the current study is the second time we report that olive oil effectively increased 25(OH)D in insufficient participants only, it is worth noting in the current study that sufficient participant numbers were smaller in the olive oil group compared with the other vitamin D groups and their baseline 25(OH)D

Table 3. Mean 25(OH)D concentrations at baseline, post-intervention and change from baseline to post and mean 25(OH)D concentrations at baseline and post-intervention in vitamin D-sufficient and -insufficient participants

		Olive		Coconut		Supplement		Placebo		RM GLM ANCOVA (*P*)		
		n 15		*n* 15		*n* 17		*n* 16				
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Time	Treatment	Time × treatment
Total	Baseline 25(OH)D (nmol/l)	63·6	23·4	61·5	19·7	56·5	21·5	60·2	18·1	0·18	0·47	< 0·001
	Post 25(OH)D (nmol/l)	69·0	13·8	71·7	12·6	72·2	14·3	56·3	18·6			
	Change 25(OH)D (nmol/l)	5·4	3·7	10·3	2·9	15·7	3·8	–3·9	1·4		< 0·001	
Sufficient	Baseline 25(OH)D (nmol/l)	77·3	4·2	68·7	3·8	69·2	4·0	70·4	4·0	0·46	0·48	0·004
	Post 25(OH)D (nmol/l)	74·2	3·8	74·9	3·4	75·8	3·6	66·8	3·6			
Insufficient	Baseline 25(OH)D (nmol/l)	36·1	4·4	32·2	5·9	32·6	4·4	38·9	4·6	< 0·001	0·30	< 0·001
	Post 25(OH)D (nmol/l)	57·3	5·4	63·2	6·8	60·8	5·1	37·4	5·3			

25(OH)D, 25-hydroxyvitamin D; RM GLM ANCOVA, repeated-measures general linear model ANCOVA.
Data are presented as mean ± standard error.
RM GLM ANCOVA, controlling for BMI at baseline, explored differences in mean 25(OH)D between groups in sufficient and insufficient participants.
GLM ANCOVA, controlling for BMI at baseline, explored differences in mean 25(OH)D between groups in sufficient and insufficient participants.
$P < 0·05$ was considered statistically significant.

Table 4. Response of biomarkers of metabolic health to intervention

	Olive		Coconut		Supplement		Placebo		RM GLM ANCOVA (*P*)		
	n 15		*n* 15		*n* 17		*n* 16				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Time	Treatment	Time × treatment
TC											
Baseline (mmol/l)	4·7	0·2	4·9	0·2	5·6	0·2	5·2	0·2	0·90	0·17	0·53
Post-intervention (mmol/l)	4·8	0·3	5·1	0·3	5·3	0·2	5·3	0·3			
HDL-cholesterol											
Baseline (mmol/l)	1·6	0·1	1·5	0·1	1·7	0·1	1·7	0·1	0·10	0·75	0·46
Post-intervention (mmol/l)	1·6	0·1	1·6	0·1	1·7	0·1	1·7	0·1			
LDL-cholesterol											
Baseline (mmol/l)	2·6	0·2	2·8	0·2	3·3	0·2	2·9	0·2	0·69	0·38	0·48
Post-intervention (mmol/l)	2·7	0·2	2·9	0·2	3·1	0·2	3·0	0·2			
TAG											
Baseline (mmol/l)	1·1	0·2	1·4	0·2	1·2	0·2	1·3	0·2	0·47	0·63	0·79
Post-intervention (mmol/l)	1·0	0·2	1·4	0·2	1·3	0·1	1·2	0·1			
Glucose											
Baseline (mmol/l)	5·3	0·1	5·4	0·1	5·4	0·1	5·6	0·1	0·73	0·27	0·21
Post-intervention (mmol/l)	5·0	0·3	5·2	0·3	5·4	0·3	6·1	0·3			
CRP											
Baseline (mg/dl)	2·9	0·5	2·1	0·5	1·6	0·5	1·8	0·5	0·79	0·39	0·49
Post-intervention (mg/dl)	3·0	0·5	1·9	0·5	1·5	0·5	1·8	0·5			

RM GLM ANCOVA, repeated-measures general linear model ANCOVA; TC, total cholesterol; CRP, C-reactive protein.
Data presented as mean ± standard error.
RM GLM ANCOVA was used to explore differences in biomarkers of metabolic health measures between groups.
$P < 0·05$ was considered statistically significant.

concentrations were higher which may impact post hoc analysis. We included coconut oil in the current study for comparison to olive oil, due to its shorter-chain SFA (~49 % lauric acid (C12:0)) and as no other vitamin D-fortified food studies have reported using coconut oil. The shorter-chain length and lack of unsaturated bonds in coconut oil may explain the larger increase in 25(OH)D compared with the olive oil group in the sufficient participants. However, we cannot compare our results to previous research as no other absorption or fortification studies include SFA. We now hypothesise that C12–18 FA with no or one double bonds may increase vitamin D absorption compared with longer-chained FA with a higher degree of unsaturation.

Baseline 25(OH)D concentration predicted 25(OH)D response to vitamin D. In a previous postprandial study, we also reported a baseline-dependent response to vitamin D and discuss the potential mechanisms driving this response based on previous literature; namely status-dependent absorption and hydroxylation changes[18,29]. A baseline-driven response is also consistently documented in intervention trials[30,31]. For example, Kaykhaei *et al.* report a larger 25(OH)D response to vitamin D in participants with baseline 25(OH)D < 25 nmol/l compared with those with higher 25(OH)D[30]. In another study, baseline 25(OH)D concentration was the strongest predictor of response to a vitamin D-fortified milk in post-menopausal women ($r = 0·5$, $P < 0·001$)[31]. Therefore, future vitamin D interventions must account for different responses between vitamin D status groups. While some trials target insufficient groups only, the effects of vitamin D strategies should be understood in all participants not just vitamin D-insufficient participants to devise optimal fortification strategies. Using these results as an example,

the coconut oil dairy drink increased 25(OH)D in all participants. Therefore, adding coconut oil to fortified dairy drinks could improve 25(OH)D in the total population, thus maintaining year-round 25(OH)D concentrations to support the extra-skeletal functions of vitamin D for all[1,32]. Therefore, small steps such as manipulating the lipid composition of foods will ensure that the population are getting as much vitamin D from foods as possible, thus increasing fortification policy effectiveness.

A 20 μg of vitamin D_3 dose brought 94 % of participants receiving vitamin D to sufficiency. Only three participants in the vitamin D groups remained insufficient post-intervention; however, they were brought close to sufficiency, with 25(OH)D increasing by 17·3–30·5 nmol/l. Our data support those from Cashman *et al.*, reporting that 13·7 μg daily maintains vitamin D sufficiency, informing the recent change to national vitamin D recommendations for older adults in Ireland[33,34]. However, it is difficult for older adults in Ireland to meet recommendations as the few foods that are fortified contain low vitamin D concentrations (0·4–2·5 μg/serving) and supplementation rates are low[25,35]. According to data modelling studies, milk and bread fortification is safe alongside a 10 μg supplement, but there are currently no safety data on higher dose fortification (> 2·5 μg/100 g)[35,36]. Our product was considered safe as no participants were at risk of vitamin D-related adverse effects (25(OH)D ≥ 125 nmol/l). However, it is worth noting that participants were asked to avoid other vitamin D supplements. Therefore, a similar 20 μg food product could be marketed for consumption in lieu of a vitamin D supplement; however, any new food product should consider the recommended serving relative to the concentration of vitamin D. Additionally, a new food product could be costly for consumers which may be a barrier for compliance as socio-economic status predicts vitamin D status in Ireland[27]. Alternatively, fortifying many lipid-based foods at a low dose could provide 20 μg daily in combination, costing only €0·11/d[37]. Government savings on fracture risk would outweigh fortification costs; thus, government investment would alleviate manufacturer and consumer financial burdens[37]. Therefore, a mandatory fortification policy, targeting a combination of frequently consumed foods with government investment, is needed urgently in Ireland.

Research on the effect of vitamin D on markers of metabolic health is mixed[38]. Observational studies report inconsistent results describing the relationship between 25(OH)D and glucose or HbA1c, as do supplementation trials in those with prediabetes[38–41]. In the current study, we observed no effect of the vitamin D groups on fasting glucose in vitamin D responders or in high or low BMI groups. Some observational studies report relationships between vitamin D status and supplementation on lipid profiles; however, RCT and meta-analyses fail to prove causality[42,43]. Our results on the effect of vitamin D on lipid biomarkers agree with the literature, but we also report that TC increased over time in those with a lower BMI. However, those in the lower BMI group had a higher percentage contribution of fat to total energy intake (data not shown), which may confound results. While the current trial reports no effect of vitamin D on biomarkers of metabolic health, it is important to note that the study was powered to determine changes in 25(OH)D only.

This study has a number of strengths and limitations which should be considered when interpreting the results. The main strength of this study is the RCT design and blinding of the dairy drink groups, but it should be noted that the supplement group was not blinded. In addition, 25(OH)D concentrations were quantified by LC-MS/MS which is the current gold standard for measuring vitamin D status; therefore, our results can be compared with other vitamin D studies using LC-MS/MS quantification. In Ireland, vitamin D can be synthesised cutaneously from April to September. Thus, the effect of cutaneous vitamin D synthesis was minimized, and participants were excluded if they were travelling abroad during the study period. High-quality dietary data were collected via 4-d food diary, which is the current gold standard. We were therefore able to rule out any dietary influences by checking if nutrient intakes were covariates in the analysis. The main limitation of this study is that participant numbers were reduced due to the COVID-19 pandemic; however, the numbers in each vitamin D group met the power requirement for comparison to the placebo control dairy drink group. Lastly, there was a large variation in baseline 25(OH)D concentrations across participants. We split participants into status groups to mediate this effect; however, a large distribution remained in these groups.

Vitamin D sufficiency is essential for skeletal and metabolic health[1]. Food fortification supports vitamin D sufficiency by contributing to vitamin D intakes. The current study demonstrates that an olive or coconut oil-based vitamin D-fortified dairy drink is equally as effective at increasing 25(OH)D as a vitamin D supplement in vitamin D-insufficient middle-aged and older adults. However, only a coconut oil vitamin D dairy drink is equally as effective as a vitamin D supplement in vitamin D-sufficient middle-aged and older adults. This is important as vitamin D fortification of lipid containing foods may be used in lieu of supplementation in older adults when supplementation adherence is low or for individuals with dysphagia. In addition, manipulating the lipid composition of fortified foods can target improved vitamin D status in the entire population to reduce seasonal 25(OH)D fluctuations and risk of extra-skeletal diseases. This research also supports previous findings that baseline 25(OH)D predicts response to vitamin D, and that this response differs in vitamin D-sufficient and -insufficient groups. Before a lipid-altered vitamin D-fortified food is developed, larger-scale research is needed on the potential effects of these lipids on 25(OH)D concentrations and metabolic health, particularly if incorporating lipids with a high proportion of SFA. To conclude, our findings support the use of coconut or olive oil as the lipid component of a fortified food as an alternative to vitamin D supplementation in vitamin D-sufficient and -insufficient groups, respectively.

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