No independent or combined effects of vitamin D and conjugated linoleic acids on muscle protein synthesis in older adults: a randomized, double-blind, placebo-controlled clinical trial

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ABSTRACT

Background: Aging is associated with skeletal muscle anabolic resistance (i.e., reduced muscle protein synthesis during anabolic conditions such as hyperaminoacidemia). The results from studies conducted in cell culture systems and animals suggest that both vitamin D and conjugated linoleic acids (CLAs) stimulate muscle protein synthesis.

Objectives: To conduct a randomized, double-blind, placebo-controlled clinical trial to determine the independent and combined effects of dietary vitamin D and CLA supplementation on myofibrillar protein synthesis rates in sedentary older adults.

Methods: Thirty-two sedentary, older adults were randomized to receive either: *1*) 2000 IU vitamin D-3 (Vit D) per day; *2*) 4000 mg CLA per day; *3*) both Vit D (2000 IU/d) and CLA (4000 mg/d); or *4*) placebo for 8 wk. Myofibrillar protein synthesis rates were evaluated by using intravenous [ring-$^2H_5$]phenylalanine infusion in conjunction with muscle biopsies during basal, postabsorptive conditions and during combined amino acid and insulin infusion before and after the supplementation period.

Results: Before the intervention, basal myofibrillar protein synthesis rates were not different among groups (Placebo: 0.033 ± 0.003; Vit D: 0.034 ± 0.002; CLA: 0.029 ± 0.005; Vit D + CLA: 0.038 ± 0.005 %·h-1), and hyperinsulinemia–hyperaminoacidemia increased myofibrillar protein synthesis rates by ∼35%. Compared with placebo, neither Vit D nor CLA nor combined Vit D + CLA supplementation affected the basal myofibrillar protein synthesis rates (placebo: 0.040 ± 0.004%/h; Vit D: 0.044 ± 0.006%/h; CLA: 0.039 ± 0.006%/h; Vit D + CLA: 0.040 ± 0.007%/h) or the hyperinsulinemia–hyperaminoacidemia–induced increase in myofibrillar protein synthesis (percentage increase from basal before and after the interventions: placebo, 30 ± 11 and 36 ± 11; Vit D, 38 ± 8 and 34 ± 10; CLA, 50 ± 14 and 51 ± 16; Vit D + CLA, 29 ± 15 and 35 ± 8).

Conclusions: Vitamin D and/or CLA supplementation, at the doses provided in our study, does not have muscle anabolic effects in sedentary older adults. The study was registered at clinicaltrials.gov (NCT03115775). *Am J Clin Nutr* 2020;112:1382–1389.

Keywords: vitamin D, conjugated linoleic acids, aging, sarcopenia, skeletal muscle, dietary supplements, protein synthesis

Introduction

Aging is associated with a progressive decline in muscle mass (1, 2), which is at least partly due to age-associated anabolic resistance (i.e., reduced muscle protein synthesis during anabolic conditions such as hyperaminoacidemia) (3–8). The results from studies conducted in cell culture systems and animals suggest that both vitamin D and conjugated linoleic acids (CLAs) can increase muscle protein synthesis and attenuate or even prevent the age-associated decline in muscle mass. First, vitamin D enhanced the stimulating effect of leucine and insulin on protein synthesis in murine C2C12 myotubes in a dose-dependent manner (9). Second, mice with deletion of the vitamin D receptor in myocytes had ∼10% lower muscle mass compared with controls (10), and dietary vitamin D depletion decreased the rate of muscle protein synthesis by ∼40% in healthy old rats (11). Third, adding CLA to the diet of healthy old mice and rats increased the muscle protein synthesis rate (12) and prevented the age-associated decline in skeletal muscle mass (13, 14). Although the mechanisms responsible for the anabolic effects of vitamin D and CLA are unclear, they are likely different, and therefore most likely additive, because CLA increased the basal rate of muscle protein synthesis only (12), whereas vitamin D increased

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Data described in the manuscript, code book, and analytic code will be made available upon request.

Supplemental Figures 1 and 2 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: BSA, body surface area; CLA, conjugated linoleic acid; FFM, fat-free mass; FSR, fractional synthesis rate, TTR, tracer-to-tracee ratio; Vit D, vitamin D (intervention).

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the basal rate of muscle protein synthesis and augmented the amino acid–induced increase in muscle protein synthesis rate (9, 11). The effects of vitamin D and CLA on muscle protein turnover in people are unknown, and the results from studies that evaluated the effect of vitamin D on muscle mass are equivocal, most likely because they used a cross-sectional study design and/or short-term interventions (15–28), which makes it difficult to detect small changes in muscle mass. However, treatment-induced changes in the rate of muscle protein synthesis that cause an increase in muscle mass typically occur quickly and are readily detectable (6, 29–31). To fill this gap, we conducted a randomized, double-blind, placebo-controlled clinical trial to determine the independent and combined effects of dietary vitamin D and CLA supplementation on the rate of muscle protein synthesis in older adults. Participants received either:*1*) 2000 IU vitamin D-3 (Vit D)/d; *2*) 4000 mg CLA/d; *3*) both Vit D (2000 IU/d) and CLA (4000 mg/d); or *4*) placebo for 8 wk. Myofibrillar protein synthesis rates were evaluated by using an intravenous [ring-$^2H_5$]phenylalanine infusion in conjunction with muscle biopsies during basal, postabsorptive conditions and during combined amino acid and insulin infusion before and after 8 wk of consuming the supplements.

Methods

Study participants

Participant recruitment and flow is shown in **Supplemental Figure 1**. A total of 70 men and women were assessed for eligibility and 32 completed this randomized, double-blind, placebo-controlled trial between July 2017 and July 2019. All participants were considered in good health (i.e., no evidence of significant cardiovascular disease or organ dysfunction) after completing a comprehensive medical evaluation, which included a health history and physical examination and standard blood tests. Furthermore, all participants fulfilled the following inclusion criteria: ≥60 and ≤85 y old, BMI ≥18.5 or ≤35 kg/m^2, stable body weight, not engaged in an exercise program for ≥3 consecutive months before starting the study, and suboptimal serum 25-hydroxyvitamin D concentration, defined as <35 ng/mL (32–34). None of the participants used tobacco products, or consumed excessive amounts of alcohol (>1 drink/d), or took dietary supplements or medications that could affect muscle protein metabolism or were incompatible with the study procedures (e.g., fish oil, anticoagulants). Participants' total body fat mass and fat-free mass (FFM) were measured by using DXA (Lunar iDXA; GE Healthcare). Written, informed consent was obtained from all participants before their participation in the study, which was approved by the Human Research Protection Office and the Clinical Research Unit Advisory Committee at Washington University School of Medicine in St Louis, MO, and registered on clinicaltrials.gov (NCT03115775).

Experimental protocol

Each participant completed 2 stable isotope-labeled tracer infusion studies to determine the effect of the interventions on the myofibrillar protein synthesis rate during basal, postabsorptive conditions and during combined amino acid and insulin infusion. The first study was performed before starting the intervention; the second took place after 8 wk of dietary supplementation with either: *1*) 2000 IU Vit D/d; *2*) 4000 mg CLA (Tonalin FFA 80) per day; *3*) both Vit D (2000 IU/d) and CLA (4000 mg/d); or *4*) placebo (corn oil). Participants were randomly assigned to the groups by the clinical research coordinator using a computerized centralized randomization scheme before baseline testing. All key study personnel and participants were blinded to the treatments. All supplements were packaged in identical-looking capsules, and were donated by BASF SE. Compliance was evaluated by pill count; in addition, we assessed changes in serum vitamin D concentration and plasma triglyceride fatty acid composition.

Participants were instructed to adhere to their usual diet and to refrain from vigorous physical activities for ≥3 d before testing. On the evening before the metabolic study, participants were admitted to the Clinical Translational Research Unit at Washington University School of Medicine. At 20:00, participants' hand grip strength was measured by using a Jamar hydraulic dynamometer (Patterson Medical); immediately thereafter, the participants consumed a standard meal and then rested in bed and fasted (except for water) until completion of the study the next day. At ∼06:00 on the following morning, a cannula was inserted into an antecubital vein for the infusion of [ring-$^2H_5$]phenylalanine (Cambridge Isotope Laboratories Inc) to measure the rate of myofibrillar protein synthesis; another cannula was inserted into a vein of the contralateral hand, which was warmed to 50°C for blood sampling. At ∼06:45, a primed, constant infusion of [ring-$^2H_5$]phenylalanine (priming dose: 7.9 μmol/kg FFM; infusion rate: 0.07 μmol/kg FFM/min) was started and maintained for 7 h. Four hours after the start of the tracer infusion, a hyperaminoacidemic–hyperinsulinemic clamp was started and maintained for 3 h. Human insulin (Novolin R; Novo Nordisk) was infused at a rate of 20 mU/m^2 body surface area (BSA)·per minute (initiated with priming doses of 80 mU/m^2 BSA/min for 5 min and then 40 mU/m^2 BSA/min for an additional 5 min) and Travasol 10% (Baxter) was infused at a rate of 105 mg amino acids/kg FFM/h (priming dose: 35 mg amino acids/kg FFM) to raise plasma insulin and amino acid concentrations to within the range normally seen after meal consumption (35–37). Euglycemia (blood glucose concentration of ∼100 mg/dL) was maintained during the clamp procedure by variable-rate infusion of 20% dextrose (Baxter). To minimize changes in plasma phenylalanine isotopic enrichment during the clamp due to the increased amino acid rate of appearance in plasma, the [ring-$^2H_5$]phenylalanine infusion rate was increased to 0.14 μmol/kg FFM/min during the clamp.

Blood samples were obtained before beginning the tracer infusions and at 60, 120, 180, 210, 220, 230, 240, 300, 360, 390, 400, 410, and 420 min to determine the labeling of phenylalanine in plasma and plasma glucose, amino acid, and insulin concentrations. Additional blood was obtained every 10 min during the clamp to monitor plasma glucose concentration. Muscle tissue (∼100 mg) was obtained under local anesthesia (lidocaine, 2%) from the vastus lateralis portion of the quadriceps femoris by using a Tilley–Henkel forceps at 60 min and 240 min (to determine the basal myofibrillar protein synthesis rate) and at 420 min, that is, 3 h after starting the clamp procedure (to determine the myofibrillar protein synthesis rate during

hyperaminoacidemia–hyperinsulinemia). The first and second biopsies were obtained from the same incision but the forceps was directed in a proximal and distal direction, respectively, so that the 2 biopsies were collected ∼5–10 cm apart. The third biopsy was obtained from the contralateral leg. Muscle samples were immediately washed in ice-cold saline solution (0.9% NaCl), snap frozen in liquid nitrogen, and stored at –80°C until further analysis.

Sample processing and analyses

Plasma glucose concentration was measured on an automated glucose analyzer (Yellow Spring Instruments). Plasma insulin concentration was determined by using an immunoassay (Elecsys; Roche Diagnostics). Plasma amino acid concentrations, phenylalanine tracer-to-tracee ratios (TTR), and plasma triglyceride fatty acid composition were determined by using GC-MS (6, 38, 39). To determine phenylalanine labeling in myofibrillar proteins, frozen muscle samples (∼50 mg) were homogenized in 10× w/v of cell lysis buffer (Cell Signaling, #9803) containing 1:100× protease and phosphatase inhibitor cocktail (Cell Signaling, #5870 and #5871, respectively); samples were heated to 50°C with 0.3M NaOH for 30 min and then centrifuged (10,000 × *g* for 5 min) and the supernatants transferred to glass tubes containing 1M perchloric acid (PCA) to precipitate myofibrillar proteins, which were hydrolyzed overnight with 6N HCl. Amino acids in the protein hydrolysate were purified on cation-exchange columns (Dowex 50W-X8–200, Millipore/Sigma, #217514), and the tertiary ButylDiMethylSilyl (t-BDMS) derivative of phenylalanine prepared to determine its TTR by GC-MS analysis (38, 40).

Calculations

The muscle protein fractional synthesis rate (FSR) was calculated from the rate of incorporation of [ring-$^2H_5$]phenylalanine into muscle protein using a standard precursor-product model as follows: $FSR = \Delta E_{protein}/E_{plasma} \times 1/t \times 100$, where $\Delta E_{protein}$ is the change in protein-bound phenylalanine enrichment between 2 consecutive biopsies, E_{plasma} is the average free phenylalanine TTR in plasma, and t is the time between biopsies. We used the average plasma phenylalanine labeling between 60 and 240 min (basal) and 270 and 420 min (clamp) as surrogate for the phenylalanine labeling in the precursor pool (i.e., aminoacyl-tRNA) (41, 42).

Statistical analysis

Data analysis.

Statistical analysis was performed by using GraphPad prism 8 (GraphPad Software) and RStudio version 1.2.1335 (RStudio, Inc). After normal data distribution was confirmed, 1-factor ANOVA was performed to examine whether differences among groups existed in subject characteristics at baseline. Repeated measures ANOVA with group and condition (basal compared with clamp) as factors were used to compare myofibrillar protein FSRs, the primary study outcome, and other metabolic outcomes (e.g., plasma glucose, insulin, and amino acid concentrations) among groups at baseline (before the intervention). ANCOVA with the pretreatment value as covariate was used to evaluate treatment effects on study outcomes (e.g., myofibrillar protein synthesis rates during basal conditions). A P value ≤ 0.05 was considered statistically significant. Data are presented as means ± SEM unless otherwise noted.

Sample size estimation.

In a study we conducted to evaluate the effect of fish oil–derived n–3 PUFA supplementation on the rate of muscle protein synthesis in older adults by using the same study design as in the present study (6), the muscle protein synthesis rates at baseline in the intervention and control groups were 0.036 ± 0.008 and 0.029 ± 0.013%/h, respectively, during basal conditions, and 0.051 ± 0.017 and 0.047 ± 0.018%/h, respectively, during hyperinsulinemia–hyperaminoacidemia. The n–3 PUFA treatment effect during hyperinsulinemia–hyperaminoacidemia was 0.022%/h. Assuming the larger SD during both basal conditions and during hyperinsulinemia-hyperaminoacidemia (0.013%/h and 0.018%/h, respectively), a power ≥0.8, and a significance cut-off value of $\alpha \leq 0.05$, we estimated that we could detect a 0.018%/h increase during basal conditions and a 0.025%/h increase during insulin and amino acid infusion with 8 subjects per group. Assuming the smaller SDs (0.008%/h and 0.017%/h), we estimated that we could detect increases of 0.011%/h and 0.023%/h during basal conditions and during hyperinsulinemia–hyperaminoacidemia, respectively, with 8 subjects per group. The power to detect main effects of Vit D or CLA treatment (i.e., Vit D alone and combined Vit D + CLA compared with placebo or CLA alone, and combined Vit D + CLA compared with placebo) exceeds the power to detect treatment effects in each of the groups because of the larger samples size ($n = 8 + 8 = 16$).

Results

Subject characteristics

Participants in the 4 groups were matched on age, sex, body weight, and body composition (**Table 1**). Basic metabolic health status and serum vitamin D concentration were not different among groups (Table 1). The calendar month when participants started the interventions, which could affect the vitamin D status due to seasonal differences in exposure to sunlight, was not different among the 4 groups (Vit D: 6.6 ± 1.1; CLA: 5.7 ± 1.2; combined Vit D + CLA: 6.5 ± 1.2; placebo: 6.5 ± 1.2, where January = 1 and December = 12).

Compliance with treatment and biomarkers of supplement intake

Average compliance, as judged by the leftover pill count, was 96 ± 2% in the Vit D group, 99 ± 1% in the CLA group, 95 ± 4% in the combined Vit D + CLA group, and 96 ± 5% in the placebo group. The serum vitamin D concentration increased by ∼25% in the Vit D alone and combined Vit D + CLA groups and slightly decreased in the CLA alone and placebo groups (**Figure 1**). The contribution of linoleic acid to total plasma triglyceride fatty acid content increased in the CLA alone and combined Vit D + CLA

TABLE 1 Subjects' age, body weight, body composition, and plasma glucose, lipid, and vitamin D concentrations at baseline, before starting the interventions[1]

	Placebo	Vit D	CLA	Vit D + CLA
Men/women	3/5	3/6	4/3	3/5
Race (C/AA/other)	8/0/0	8/1/0	7/0/0	8/0/0
Age, y	69 ± 1	69 ± 2	67 ± 2	70 ± 2
BMI, kg/m^2	28 ± 2	28 ± 1	27 ± 2	28 ± 2
Weight, kg	79 ± 5	78 ± 4	79 ± 4	82 ± 5
Fat-free mass, kg	48 ± 3	46 ± 3	50 ± 3	48 ± 4
Body fat, %	38 ± 4	41 ± 2	36 ± 4	40 ± 2
Glucose, mg/dL	100 ± 4	101 ± 3	98 ± 4	95 ± 3
Triglycerides, mg/dL	95 ± 13	104 ± 11	119 ± 25	107 ± 12
LDL-cholesterol, mg/dL	120 ± 14	121 ± 9	101 ± 14	111 ± 6
HDL-cholesterol, mg/dL	66 ± 6	65 ± 4	54 ± 7	56 ± 5
Vitamin D-3, ng/mL	29 ± 1	23 ± 2	27 ± 2	23 ± 3

[1]Data are mean ± SEM. One-factor ANOVA was used to compare outcomes among groups. AA, African American; C, Caucasian; CLA, conjugated linoleic acid; Vit D, vitamin D.

groups but remained unchanged in the Vit D alone and placebo groups (Figure 1).

Plasma glucose, insulin, and amino acid concentrations and enrichment

Basal plasma glucose, insulin, and amino acid concentrations were not different among the groups before starting the intervention (**Table 2**). During the hyperinsulinemic–euglycemic clamp with concomitant amino acid infusion, plasma glucose was successfully maintained at ~100 mg/dL, insulin increased from ~10 to ~50 mU/L, and amino acids increased from ~2300 to ~3200 μM with no differences among groups (Table 2). Glucose, insulin, and amino acid concentrations were not affected by the treatments (Table 2). Plasma phenylalanine enrichment was stable from the time of the first muscle biopsy to the time of the third muscle biopsy, both before and after the interventions (**Supplemental Figure 2**).

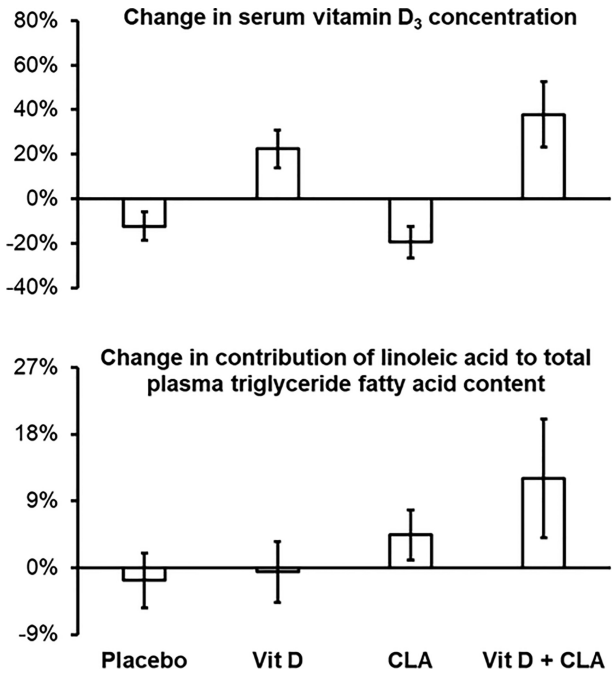

FIGURE 1 Treatment-induced changes in serum vitamin D-3 concentration (top) and contribution of linoleic acid to total plasma triglyceride fatty acid content (bottom) in the placebo (n = 8), vitamin D (Vit D, n = 9), conjugated linoleic acid (CLA, n = 7), and combined Vit D + CLA (n = 8) supplementation groups. Values are means ± SEM.

Myofibrillar protein synthesis rate

Basal myofibrillar protein synthesis rates before the interventions were not different among groups (placebo: 0.033 ± 0.003%/h; Vit D: 0.034 ± 0.002%/h; CLA: 0.029 ± 0.005%/h; and Vit D + CLA: 0.038 ± 0.005%/h); hyperinsulinemia–hyperaminoacidemia increased myofibrillar protein synthesis rates by ~35% without a difference among the groups (placebo: 0.042 ± 0.005%/h; Vit D: 0.048 ± 0.006%/h; CLA: 0.048 ± 0.005%/h; and Vit D + CLA: 0.047 ± 0.005%/h) (**Figure 2**). Compared with placebo, the treatments had no effect on basal myofibrillar protein synthesis rates (placebo: 0.040 ± 0.004%/h; Vit D: 0.044 ± 0.006%/h; CLA: 0.039 ± 0.006%/h; and Vit D + CLA: 0.040 ± 0.007%/h), myofibrillar protein synthesis rates during hyperinsulinemia–hyperaminoacidemia (placebo: 0.056 ± 0.007%/h; Vit D: 0.056 ± 0.005%/h; CLA: 0.057 ± 0.009%/h; and Vit D + CLA: 0.055 ± 0.011%/h), or the hyperinsulinemia–hyperaminoacidemia–induced increase in myofibrillar protein synthesis rates (percentage increase from basal before and after the interventions: placebo, 30 ± 11 and 36 ± 11; Vit D, 38 ± 8 and 34 ± 10; CLA, 50 ± 14 and 51 ± 16; Vit D + CLA, 29 ± 15 and 35 ± 8) (Figure 2). It is unlikely that our study lacked sufficient statistical power to detect beneficial treatment effects because there was no trend (neither statistically nor numerically) for increased myofibrillar protein synthesis rates in any of the treatment groups compared with the placebo group; moreover, we did not observe a treatment effect even when we combined the Vit D alone and the Vit D + CLA groups or the CLA alone and the Vit D + CLA groups compared with those that did not receive Vit D or CLA, respectively (data not shown).

TABLE 2 Plasma glucose, insulin, and amino acid concentrations during basal conditions and during the hyperinsulinemic euglycemic clamp with concomitant amino acid infusion before and after the interventions[1]

	Before		After	
	Basal	Clamp	Basal	Clamp
Glucose, mg/dL				
Placebo	96 ± 4	99 ± 2	95 ± 4	99 ± 2
Vit D	96 ± 2	99 ± 1	95 ± 3	97 ± 1
CLA	95 ± 3	98 ± 2	95 ± 4	100 ± 3
Vit D + CLA	94 ± 2	96 ± 3	94 ± 3	103 ± 3
Insulin, mU/L				
Placebo	8.8 ± 1.7	50 ± 6*	7.8 ± 1.1	50 ± 7*
Vit D	9.2 ± 1.0	46 ± 2*	10.0 ± 1.1	48 ± 4*
CLA	10.4 ± 3.6	47 ± 6*	9.8 ± 3.3	45 ± 5*
Vit D + CLA	9.7 ± 1.2	52 ± 6*	9.3 ± 1.2	48 ± 7*
Total amino acids, μM				
Placebo	2400 ± 275	3197 ± 185*	2327 ± 172	3188 ± 137*
Vit D	2242 ± 138	3358 ± 195*	2330 ± 141	3313 ± 181*
CLA	2212 ± 76	3154 ± 110*	2347 ± 95	3261 ± 114*
Vit D + CLA	2329 ± 128	3281 ± 142*	2537 ± 230	3380 ± 206*
Essential amino acids, μM				
Placebo	1108 ± 153	1568 ± 96*	1086 ± 77	1586 ± 73*
Vit D	1081 ± 69	1694 ± 113*	1127 ± 76	1710 ± 112*
CLA	1061 ± 40	1600 ± 60*	1123 ± 38	1672 ± 64*
Vit D + CLA	1121 ± 68	1672 ± 85*	1158 ± 115	1676 ± 110*
Phenylalanine, μM				
Placebo	113 ± 9	210 ± 11*	117 ± 11	211 ± 14*
Vit D	101 ± 5	190 ± 9*	109 ± 5	193 ± 10*
CLA	104 ± 6	198 ± 12*	109 ± 6	206 ± 9*
Vit D + CLA	109 ± 10	196 ± 10*	109 ± 8	201 ± 8*

[1]Data are mean ± SEM. ∗Different from corresponding basal value ($p<0.05$). Repeated measures ANOVA with group and condition (basal and clamp) as factors were used to evaluate the effect of hyperaminoacidemia–hyperinsulinemia among the groups before and after the interventions. ANCOVA with the pretreatment value as covariate was used to evaluate treatment effects. No between group differences were observed (all $P > 0.05$). Sample sizes: placebo, $n = 8$; Vit D, $n = 9$; CLA, $n = 7$; Vit D + CLA, $n = 8$. CLA, conjugated linoleic acid; Vit D, vitamin D.

Hand-grip strength

Hand-grip strength was not different among groups at baseline and did not change during the interventions (**Figure 3**).

Discussion

The age-associated decline in muscle mass is a significant public health problem because it can negatively affect activities of daily living and quality of life (1, 2). Interventions to prevent and treat the age-associated decline in muscle mass focus on increasing the stimulatory effect of amino acids on myofibrillar protein synthesis to overcome the anabolic resistance of skeletal muscle in older adults (3–8). Studies conducted in cultured myocytes and in vivo in animals suggest both vitamin D and CLA stimulate muscle protein synthesis and increase muscle mass (9–14). We evaluated the independent and combined effects of dietary vitamin D (2000 IU/d) and CLA (4000 mg/d) supplementation on myofibrillar protein synthesis rates during basal, postabsorptive conditions and during combined amino acid and insulin infusion in sedentary older adults. We found that, compared with placebo treatment, neither vitamin D nor CLA treatment alone or combined vitamin D + CLA treatment increased the myofibrillar protein synthesis rates. Therefore, we conclude that neither vitamin D nor CLA have muscle anabolic effects in sedentary older adults at the doses used in our study.

We studied sedentary older adults who did not engage in any physical activities, except normal activities of daily living, because the majority of older adults do not regularly engage in more strenuous physical activities and/or structured exercise programs. It is possible that a positive treatment effect on muscle protein synthesis would have been observed in exercising muscles. It is also possible, but unlikely, that we did not observe a treatment effect in our study because of the specific population we studied. Even though none of our participants had vitamin D deficiency (serum vitamin D concentration <12 ng/mL) and not all had insufficiency (<20 ng/mL) according to guidelines published by the Institute of Medicine (43), we studied older adults with serum vitamin D concentrations (grand mean: 25.6 ± 1.1 ng/mL) that are considered insufficient according to guidelines published by the American Geriatrics Society and the Endocrine Society (33, 34). In fact, 42% of our participants who received vitamin D treatment even had vitamin D concentrations that are considered insufficient according to the more stringent (<20 ng/mL) guidelines published by the Institute of Medicine (43). Furthermore, we found no differences in baseline (before intervention) rates of muscle protein synthesis in participants with vitamin D insufficiency (serum vitamin D <20 ng/mL) compared with those with serum vitamin D concentrations >20 ng/mL (basal conditions: 0.035 ± 0.001 compared with 0.034 ± 0.002%/h; combined amino acid and insulin infusion: 0.050 ± 0.001 compared

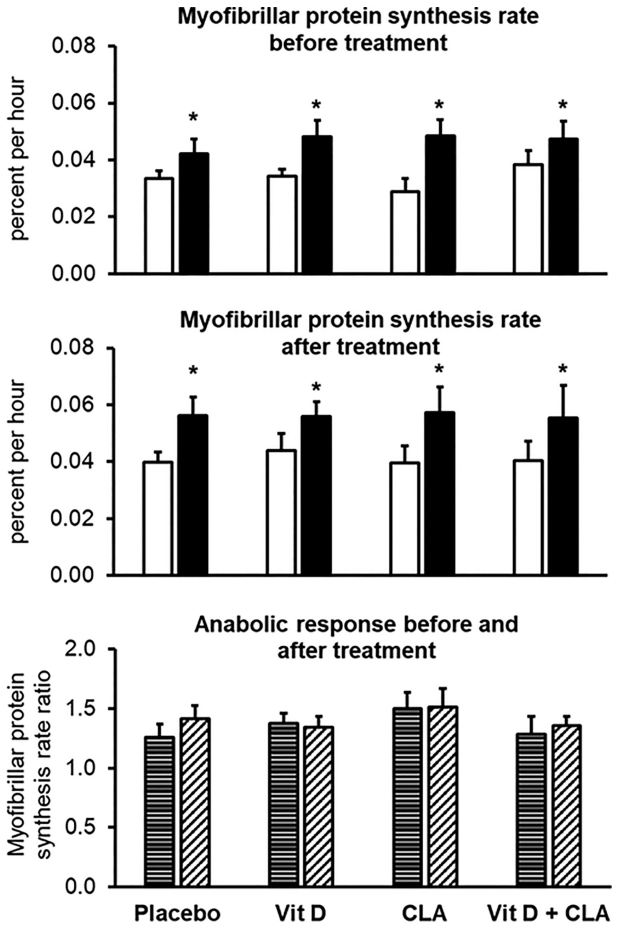

FIGURE 2 Myofibrillar protein synthesis rates during basal conditions (white bars) and during the hyperinsulinemic–euglycemic clamp procedure with concomitant amino acid infusion (black bars) before (top) and after (middle) the interventions, and the anabolic response (bottom), assessed as the ratio of myofibrillar protein synthesis rate during the hyperinsulinemic–euglycemic clamp to the myofibrillar protein synthesis rate during basal conditions, before (horizontally striped bars) and after (diagonally striped bars) the interventions in the placebo ($n = 8$), vitamin D (Vit D, $n = 9$), conjugated linoleic acid (CLA, $n = 7$), and combined Vit D + CLA ($n = 8$) supplementation groups. Values are mean ± SEM. Repeated measures ANOVA with group and condition (basal and clamp) as factors was used to evaluate the effect of hyperaminoacidemia–hyperinsulinemia among the groups before and after the interventions. ∗Different from corresponding basal value, $P < 0.05$. ANCOVA with the pretreatment value as covariate was used to evaluate treatment effects. No differences in treatment responses were observed among groups.

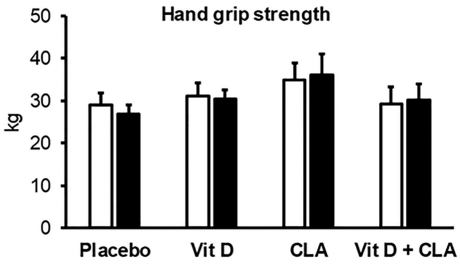

FIGURE 3 Hand-grip strength before (white bars) and after (black bars) the interventions in the placebo ($n = 8$), vitamin D (Vit D, $n = 9$), conjugated linoleic acid (CLA, $n = 7$), and combined Vit D + CLA ($n = 8$) supplementation groups. Values are mean ± SEM. One-factor ANOVA was used to compare outcomes among groups at baseline. ANCOVA with the pretreatment value as covariate was used to evaluate treatment effects. No differences in treatment responses were observed among groups.

with 0.046 ± 0.003%/h, respectively). We also found no effect of treatment with vitamin D in participants with vitamin D insufficiency (Vit D alone and Vit D + CLA groups combined) at baseline (basal conditions: 0.034 ± 0.003 compared with 0.036 ± 0.004%/h; combined amino acid and insulin infusion: 0.048 ± 0.006 compared with 0.048 ± 0.005%/h, before and after treatment, respectively), even though serum vitamin D increased by 53 ± 14%. It is unlikely that the duration of the interventions in our study was too short. We chose the 8-wk intervention period because we (6, 29) and others (30, 31) found known anabolic treatments, such as resistance exercise, testosterone, and fish oil–derived n–3 PUFAs, increase the rate of muscle protein synthesis within ≤8 wk.

The Institute of Medicine's recommended daily intake for vitamin D, which is expected to achieve a serum vitamin D concentration of ≥20 ng/mL in ≥97.5% of the population, is 600 IU/d for 18–70-y-old adults and 800 IU/d for those aged >70 y (43). The American Geriatrics Society recommends a daily intake of 4000 IU, including ≥1000 IU/d from dietary vitamin D supplements (33). The supplements used in our study provided 2000 IU vitamin D/d and resulted in a ∼45% increase in serum vitamin D in study participants who received vitamin D compared with those who did not. The increase in serum vitamin D we observed is consistent with the increases observed in other studies that provided similar doses of vitamin D (16, 44). These findings demonstrate that our participants were compliant with the treatment, which we also confirmed by pill count, and support the notion that our participants' baseline vitamin D status had not reached a ceiling above which there would be no further increase in body vitamin D stores or biological effects. There are no guidelines for CLA intake. We provided 4000 mg CLA, which is about 10 times as much as typically consumed in the diet and represents an amount that was found to have biological effects (i.e., reduce body fat) in randomized clinical trials (45–47).

It is possible, but unlikely, that we did not observe a treatment effect because our participants did not demonstrate age-associated anabolic resistance in muscle. We used the same experimental protocol we had previously used to evaluate the effect of fish oil–derived n–3 PUFAs on the rate of muscle protein synthesis (6) and found that the combined amino acid and insulin infusion in the present study increased the myofibrillar protein synthesis rate by ∼45% above basal values. This is consistent with the results from our previous study (6) and demonstrates anabolic resistance because the same amino acid and insulin infusion protocol approximately doubled the muscle protein synthesis rate in healthy young adults (6). We also carefully considered the amino acid and insulin infusion rate and chose a dose that submaximally stimulates the muscle protein synthesis rate (48) to avoid a ceiling effect, which could mask a beneficial effect of treatment.

The results from our study are consistent with the results from most randomized controlled trials that evaluated the effect of vitamin D supplementation on muscle mass. The authors of a systematic review and meta-analysis published in 2014 found no effect of vitamin D on muscle mass (15). In more recent prospective, randomized, controlled trials that lasted 3–6 mo,

vitamin D in doses that ranged from 400 IU to 2000 IU/d or 40,000 IU/wk, also did not increase muscle mass compared with placebo (16, 49, 50). In addition, a secondary analysis of data from a randomized controlled trial found that a monthly dose of 50,000 IU (equivalent to ∼ 1700 IU/d) of vitamin D for 12 mo did not affect muscle mass (assessed as thickness and cross-sectional area by using ultrasound) in 50–79-y-old men and women with low serum vitamin D (<25 ng/mL) (51). However, it was found that 10,000 IU of vitamin D consumed 3 times per week (equivalent to ∼4300 IU/d) for 6 mo increased muscle mass in a subset of lean older adults (17). In addition, an inverse association between serum vitamin D concentration and indices of muscle mass was observed in both cross-sectional and prospective observational studies (18–24, 52). However, these studies included people with vitamin deficiency (52) and the associations were often not significant when statistical adjustments for important confounding variables were made (18–21, 24). In fact, several studies found no association between vitamin D status and muscle mass or found that even people with normal muscle mass had vitamin D insufficiency (22, 25–28). The results from our study are different from those observed in animals, most likely because the animal studies compared the muscle protein synthesis rates in animals with normal vitamin D status and animals with severe vitamin D deficiency induced by using a vitamin D–depleted diet (11) or animals with muscle vitamin D receptor knock-out (10).

In summary, we conducted a double-blind, randomized, placebo-controlled clinical trial to evaluate the independent and combined effects of vitamin D and CLA supplementation on myofibrillar protein synthesis rates during basal, postabsorptive conditions and during amino acid and insulin infusion in sedentary older adults. We found neither vitamin D nor CLA nor their combination affected muscle protein synthesis rates. Therefore, we conclude that neither vitamin D nor CLA have muscle anabolic effects in sedentary older adults at the doses provided in our study.

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