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Research Article

# Nutritional Supplementation With Physical Activity Improves Muscle Composition in Mobility-Limited Older Adults, The VIVE2 Study: A Randomized, Double-Blind, Placebo-Controlled Trial

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## Abstract

**Background:** Nutritional supplementation and physical activity have been shown to positively influence muscle mass and strength in older adults. The efficacy of long-term nutritional supplementation in combination with physical activity in older adults remains unclear.
**Methods:** Mobility-limited (short physical performance battery [SPPB] ≤9) and vitamin D insufficient (serum 25(OH) D 9–24 ng/mL) older adults were recruited for this study. All subjects participated in a physical activity program. Subjects were randomized to consume a daily nutritional supplement (150 kcal, 20 g whey protein, 800 IU vitamin D, 119 mL beverage) or placebo (30 kcal, nonnutritive, 119 mL). In a prespecified secondary analysis, we examined total-body composition (dual energy X-ray absorptiometry), thigh composition (computed tomography), and muscle strength, power, and quality before and after the 6-month intervention.
**Results:** One hundred and forty-nine subjects were randomized into the study [mean (standard deviation, *SD*) age 78.5 (5.4) years; 46.3% female; mean (*SD*) short physical performance battery 7.9 (1.2); mean (*SD*) vitamin D 18.7 (6.4) ng/mL]. After the intervention period both groups demonstrated improvements in muscle strength, body composition, and thigh composition. Nutritional supplementation lead to further losses of intermuscular fat ($p = .049$) and increased normal muscle density ($p = .018$).
**Conclusions:** Six months of physical activity resulted in improvements in body composition, subcutaneous fat, intermuscular fat, and strength measures. The addition of nutritional supplementation resulted in further declines in intermuscular fat and improved muscle density compared to placebo. These results suggest nutritional supplementation provides additional benefits to mobility-limited older adults undergoing exercise training.
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**Keywords:** Exercise, Elderly, Protein, Vitamin D, Sarcopenia

Advancing age is associated with a loss of muscle mass, strength, and quality that leads to an increased risk of falls, mobility limitations, hospitalization, and death (1,2). Although the onset and progression of age-related muscular impairment is multifactorial and complex, interventional strategies that target modifiable risk factors can provide therapeutic effects in this population (3). In particular, exercise interventions have been shown to positively influence muscle mass, function, and mobility status (4). Although less is known about nutritional interventions, those which have utilized protein and/or vitamin D intake to address deficiencies in this population have shown promise (5,6). Acute studies demonstrate that the synergistic effects of protein ingestion and resistance training potentiate muscle protein synthesis (MPS) (7,8). Further, vitamin D supplementation has been shown to augment the anabolic effect of leucine and enhance certain functional outcomes in response to exercise (9,10). However, few trials have investigated the long-term efficacy of protein and vitamin D supplementation with exercise in older adults, and results have been mixed (11–15).

The age-related accumulation of intermuscular adipose tissue (IMAT) has recently emerged as a modifiable risk factor contributing to metabolic and muscular dysfunction with age (16,17). Several studies have examined the effects of calorie restriction and/or exercise on IMAT in young and middle-aged cohorts, typically leading to weight loss and decreased levels of IMAT (18,19). There is currently a paucity of literature which has aimed to determine the effects of exercise on IMAT in older adults (20,21). Moreover, certain lines of evidence suggest specific nutrients augment myogenic signaling and have the potential to promote improvements in muscle composition (22). To our knowledge, no clinical trials have investigated the effects of structured physical activity in combination with nutritional supplementation on IMAT in this population. As there are several factors contributing to the loss of muscle mass and function with age, integrated interventional strategies which target multiple aspects of age-related muscular aberrancy have the potential to maximize therapeutic outcomes in this population (3).

"The Vitality, Independence, and Vigor Study (VIVE2)" was designed to compare the effects of a low volume (119 mL) ready-to drink nutritional supplement providing 150 kcal, high amounts of whey protein (20 g) and vitamin D (800 IU) to a low-calorie placebo (30 kcal, nonnutritive, 119 mL, identical format) on adaptations to exercise in older adults with demonstrated mobility limitations and vitamin D insufficiency (9–24 ng/mL) across two field centers. The primary results from this trial suggest physical activity improves mobility and nutritional supplementation does not lead to further improvements (23). In a prespecified secondary analysis, we aimed to determine whether, compared with placebo, a high protein, high vitamin D nutritional supplement with structured physical activity induced greater changes in whole-body composition, thigh composition, and muscle strength.

## Methods

The design and recruitment methods of this study have been described elsewhere (23,24). The Tufts University Health Sciences Campus Institutional Review Board and the Regional Ethical Committee of Uppsala, Sweden, reviewed and approved the study protocol. Data management and quality control were overseen by Nestle Health Science, Vevey, Switzerland.

### Randomization

Subject randomization to the supplement or placebo groups was stratified for sex and clinical site, utilizing a block size of 10. Randomization was computer-generated and determined the order in which the supplement and placebo were assigned in each block. The group assignments based on this randomization scheme were administered by a research assistant not affiliated with this study or the study participants. Study assessors, data analysts, and investigators were blinded to group assignment.

### Interventions

#### Physical activity

Study participants were asked to attend a center-based physical activity program three times a week for 6 months. The physical activity intervention included walking, lower-extremity strength exercises, balance, and flexibility. A detailed description of the physical activity intervention has been previously described (24). For the secondary perprotocol (PP) analyses (see below), participants were considered adherent to the exercise intervention if they attended ≥60% of the exercise sessions over the 6-month intervention period.

#### Nutritional supplement

Participants were randomized to consume either a nutritional supplement or placebo, once daily over the 6-month intervention period. The supplement/placebo was consumed post-exercise on scheduled training days. The nutritional supplement contained 20 g of whey protein, 800 IU of vitamin D, 350 mg of calcium, and an assortment of other vitamins and minerals in a 119 mL, 150 kcal beverage. For a more detailed description of the nutritional supplement, please see Supplementary Table 1of the primary outcomes paper (23). The placebo was a 119 mL nonnutritive sweetened drink providing 30 kcal per serving. For a more detailed description of the placebo, please see the design and methods paper (24). Both products were developed and manufactured by Nestle Health Science, Vevey, Switzerland, and were sent directly to the study sites. For the secondary PP analyses (see below), participants were considered adherent to the nutritional component if they utilized ≥60% of their intended doses over the 6-month intervention period.

#### Serum vitamin D status

Serum 25 (OH) vitamin D was analyzed using a standard immunoassay (*DiaSorin RIA kit*; *DiaSorin*, Stillwater, MN, USA).

#### Safety

Serious adverse events were logged and monitored. Nonserious adverse events (AEs) were classified by system organ class, per the Medical Dictionary for Regulatory Activities.

### Outcomes

Outcomes assessed at baseline and 6 months included: body composition, thigh composition, muscle strength, muscle power, and muscle quality. Detailed descriptions of these measurements have been previously described (24).

#### Body composition

Whole-body and regional fat and muscle mass were assessed for this study using dual energy X-ray absorptiometry (DXA) (Hologic, Discovery A (Hologic, Bedford, MA) used at Tufts; GE Lunar (Madison, WI) used at Uppsala) (25). The DXA system generates photons at two principal energy levels (40 and 70 KeV) which allow measurement of bone mineral and soft tissue. All scans were centrally analyzed at Tufts by a single investigator in a blinded manner. Total-body mass, muscle mass, fat mass, and appendicular lean mass were derived.

#### Thigh composition

Computed tomography scans of the nondominant thigh were performed at the midpoint of the femur for each subject (Figure 1). The length of the femur was determined from a coronal scout image as the distance between the intercondylar notch and the trochanteric notch. All scans were obtained using a Siemens Somatom Scanner (Erlangen, Germany) operating at 120 KV and 100 mA. Technical factors included a slice width of 10 mm and a scanning time of 1 s. All scans were centrally analyzed at Tufts by a single investigator in a blinded manner using SliceOmatic v4.2 software (Montreal, Canada). Images were reconstructed on a 512 × 512 matrix with a 25-cm field of view.

The thigh muscle cross-sectional area (CSA) was considered the total area of nonadipose and nonbone tissue within the deep fascial plane, quantified in the range of 0–100 Hounsfield units (HU). Further, thigh muscle CSA was partitioned into low-density muscle CSA (0–34 HU) and normal-density muscle CSA (35–100 HU). This noninvasive technique has been shown to be a valid measure of intramyocellular lipid content, with lower densities representing greater levels of fat infiltration within the myocyte (26).

Adipose tissue areas were measured in the range of −190 to −30 HU. Intermuscular fat (adipocytes located between muscles) was distinguished from subcutaneous adipose tissue by manually drawing a line along the deep fascial plane. Similar methods have been previously described (17).

#### Muscle strength, power, and quality

Strength and power were determined for the knee flexors and extensors using the Biodex System 3 Isokinetic Dynamometer (Biodex Medical Systems, Shirley, NY). Isokinetic strength was measured in newton meters (Nm) and assessed at 60°/s. Muscle power was measured in watts (W) and assessed at 180°/s. These specific angular velocities were selected because of their association with functional performance and good reliability in this population (27). Muscle quality was assessed as isokinetic strength/thigh muscle CSA.

#### Subgroup analyses

Along with the intention to treat analyses (ITT), secondary PP analyses were conducted. The PP analyses included participants with adequate adherence to both the exercise and nutrition components of the intervention (see above).

#### Statistical analyses

Mean change in body composition, strength, and power measures were computed for each group along with corresponding 95% confidence intervals. Between-group differences in change in these parameters were estimated using mixed-effects linear regression analysis with statistical control for study site (as a random effect), participant sex and baseline outcome measurements. Hypothesis testing was conducted at the .05 level.

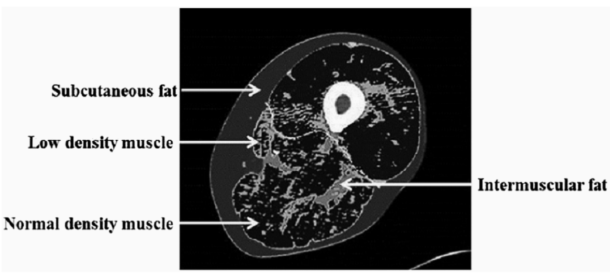


**Figure 1.** Representative computed tomography (CT) image delineating the different compartments of the thigh that were utilized in the analysis of thigh composition.

## Results

### Study Participants and Enrollment

Screening and enrollment data have been reported previously (23). Baseline characteristics were similar among the randomized groups (Table 1).

### Analytic Samples

One hundred and thirty-seven of enrolled participants had evaluable follow-up data at 6 months and 120 (60 per arm) qualified for the PP analysis.

### Intervention Adherence and Safety

The mean session attendance for physical activity was >70% in both groups and supplement/placebo intake was >85% in both groups (23). For detailed intervention adherence results, please see the primary outcomes paper (23).

### Total-Body Composition

Table 2 shows baseline and mean changes in total-body and thigh composition by study group (PP analyses). Results were very similar in both ITT and PP analyses. The remaining analyses will refer to the PP results exclusively. For ITT results, please see Supplementary Material (Supplementary Table 1). There were no substantial differences between the groups at baseline. Decreases in body mass, fat mass, and subcutaneous fat were similar between groups (Table 2). Consistent with the underlying exercise intervention, both groups exhibited statistically significant decreases in total-body fat mass, and increases in thigh muscle CSA.

### Thigh Composition

Participants in the supplement group experienced greater declines in intermuscular fat than did those in the control group (−12% compared to −5%, respectively; Figure 2). The supplement group also experienced a greater increase in normal-density muscle than control (+6% compared to +1%, respectively) and within the supplement group, there was a significant decrease in low-density muscle (Figure 3) with a trend for a treatment effect ($p$ = .08). There was also a statistical trend for a treatment effect in thigh muscle CSA ($p$ = .07).

**Table 1.** Participant Characteristics at Baseline ($N$ = 149); Mean ($SD$) or Count (%)

| | Supplement ($n$ = 74) | Placebo ($n$ = 75) |
|---|---|---|
| Age, year | 78.1 (5.8) | 76.9 (4.9) |
| Female sex | 34 (46%) | 35 (47%) |
| Body mass index, kg/m² | 27.9 (3.3) | 28.4 (3.9) |
| MMSE score | 27.3 (1.7) | 27.4 (1.9) |
| Number of medical diagnoses | 2.8 (2.5) | 2.8 (2.7) |
| 25(OH)D, ng/mL | 19.7 (6.8) | 17.7 (5.9) |
| Appendicular lean mass index, kg/m² | 7.1 (1.0) | 7.4 (1.1) |

**Table 2.** Total-Body and Thigh Composition at Baseline and Mean Changes With Intervention (PP Sample)

| | Physical activity + placebo | | Physical activity + nutritional supplementation | | |
|---|---|---|---|---|---|
| | Baseline ($n$ = 57–59) | Mean change ($n$ = 54–57) | Baseline ($n$ = 58–60) | Mean change ($n$ = 58–60) | $p$ between group |
| Body mass, kg | 79.7 ± 12.5 | −0.46 (−0.98, 0.06) | 80.4 ± 13.3 | −0.49 (−1.13, 0.14) | .93 |
| Fat mass, kg | 27.5 ± 7.8 | −0.9 (−1.25, −0.56)*** | 28.4 ± 7.8 | −0.62 (−1.09, −0.15)* | .342 |
| Lean mass, kg | 49.8 ± 10 | 0.45 (0.01, 0.89)* | 49.6 ± 9.8 | 0.13 (−0.27, 0.53) | .292 |
| Appendicular lean mass, kg | 21 ± 4.5 | 0.09 (−0.13, 0.31) | 20.8 ± 4.3 | 0.16 (−0.06, 0.38) | .66 |
| Thigh muscle CSA, cm² | 103.1 ± 23.3 | 1.51 (0.59, 2.43)** | 98.2 ± 22.9 | 3.03 (1.69, 4.38)*** | .071 |
| Normal-density muscle CSA, cm² | 77.9 ± 22.3 | 1 (−0.93, 2.93) | 72.4 ± 22.3 | 4.28 (2.42, 6.14)*** | .018 |
| Low-density muscle CSA, cm² | 25.2 ± 8.9 | 0.51 (−1, 2.02) | 25.8 ± 11.5 | −1.25 (−2.45, −0.05)* | .077 |
| Subcutaneous adipose tissue CSA, cm² | 72 ± 41.7 | −2.2 (−3.71, −0.69)** | 82.9 ± 42.9 | −2.57 (−4.91, −0.24)* | .792 |
| Intermuscular fat CSA, cm² | 5.7 ± 4.0 | −0.26 (−0.45, −0.07)** | 5.4 ± 4.6 | −0.63 (−0.93, −0.32)*** | .049 |

All baseline values are means ± *SD*s. Mean change (95% CI): *$p$ < .05, **$p$ < .01, ***$p$ < .001.

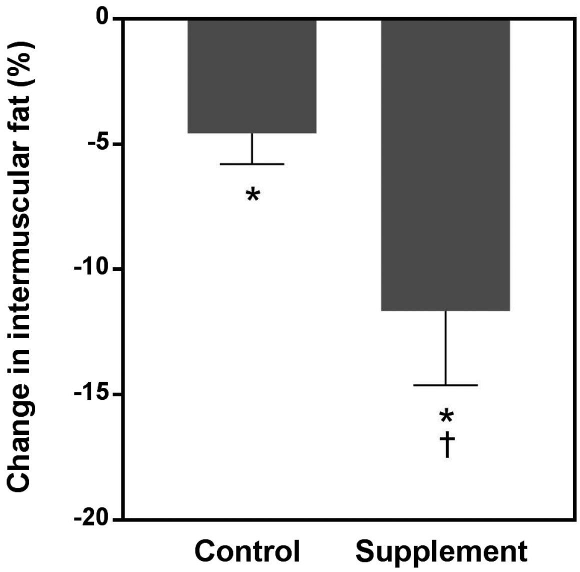


**Figure 2.** Mean percent change in intermuscular fat in the active and control groups (PP analysis). *Significant change within group compared with baseline. †Significant change between groups: $p$ = .049.

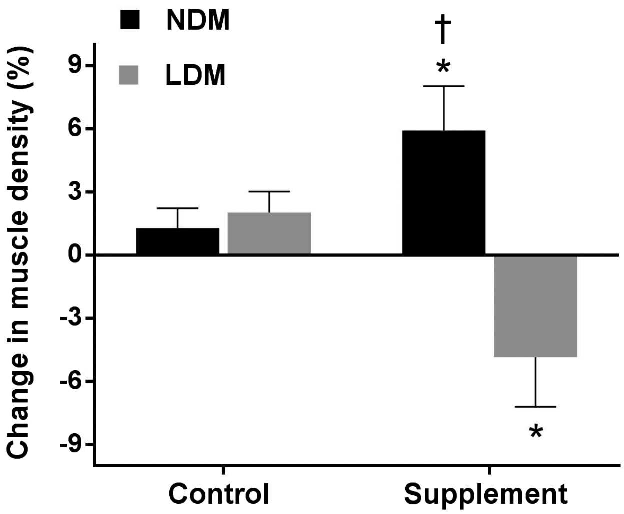


**Figure 3.** Mean percent change in low-density muscle (LDM) and normal-density muscle (NDM) in the active and control groups (PP analysis). *Significant change within group compared with baseline. †Significant change between groups: $p$ = .018.

## Muscle Strength, Power, and Quality

Table 3 shows baseline and mean changes in muscle strength, power, and quality by study group (PP analyses). At baseline, there were statistical differences between groups in muscle strength and power ($p$ < .05). Measures of knee flexor strength, power, and quality increased significantly over time in both active and control groups, but with no treatment × time effect. Results were very similar in both ITT and PP analyses.

## Discussion

Six months of structured physical activity in vitamin D deficient and mobility-limited older adults led to significant improvements in total-body composition and thigh-specific improvements in subcutaneous adipose tissue, intermuscular fat, muscle CSA, muscle strength, power, and quality. The addition of a high protein, high vitamin D nutritional supplement increased normal-density muscle and lead to greater losses of intermuscular fat. Further, in the group receiving supplementation, there was a decrease in low-density muscle and a statistical trend for a treatment effect in thigh muscle CSA.

Despite the acute benefits of high-quality protein supplementation being well established (7,8), the benefits of prolonged nutrient supplementation in older adults remain unclear, particularly in mobility-limited, sarcopenic, and frail populations (11–15). Interventional strategies which target at risk older adults prior to the onset of clinically evident disability have important clinical implications. Older adults who are able to maintain ambulation have lower rates of morbidity and mortality, and are better able to maintain their independence (28,29), in turn preserving quality of life and reducing excess cost to the health care system in this population (28). Although a recent systematic review suggests protein supplementation does not provide additional benefits to exercise in older adults (30), current reports suggest long-term, multinutrient supplementation with, or without, exercise increases muscle mass and strength in mobility-limited and sarcopenic older adults (5,11). Although our results do not support these recent findings, our participants were not malnourished, and a higher dose of protein in our nutritional supplement and/or a more robust strength training stimulus may have been needed to maximally stimulate MPS in this population (31).

It is widely accepted that levels of muscle mass and strength influence physical function in older adults (32). More recently, an

**Table 3.** Muscle Strength, Power, and Quality at Baseline and Mean Changes With Intervention (PP Sample)

| | Physical activity + placebo | | Physical activity + nutritional supplementation | | |
|---|---|---|---|---|---|
| | Baseline ($n$ = 57–59) | Mean change ($n$ = 54–57) | Baseline ($n$ = 58–60) | Mean change ($n$ = 58–60) | $p$ between group |
| Knee extensor strength, Nm† | 75.8 ± 31.3 | 0.72 (−4.36, 5.8) | 67.7 ± 27.4 | −0.21 (−5.37, 4.95) | .801 |
| Knee flexor strength, Nm† | 33.4 ± 15.4 | 9.08 (5.03, 13.14)*** | 28.7 ± 14.5 | 7.27 (3.16, 11.37)*** | .539 |
| Knee extensor power, W† | 72.7 ± 33.8 | 5.66 (−2.48, 13.8) | 59 ± 30.2 | 5.36 (−2.57, 13.29) | .958 |
| Knee flexor power, W† | 26.4 ± 21.5 | 16.73 (10.12, 23.34)*** | 15.9 ± 16.8 | 17.32 (10.9, 23.75)*** | .9 |
| Knee extensor quality[a] | 0.74 ± 0.23 | 0 (−0.05, 0.05) | 0.7 ± 0.26 | −0.04 (−0.09, 0.01) | .295 |
| Knee flexor quality[a] | 0.33 ± 0.13 | 0.09 (0.05, 0.13)*** | 0.3 ± 0.15 | 0.06 (0.01, 0.1)* | .323 |

All baseline values are means ± *SD*s. Between-group difference at baseline: †$p < .05$. Mean change (95% CI): *$p < .05$, ***$p < .001$.
[a]Nm/thigh muscle CSA.

age-related fatty infiltration of the musculature has gained clinical interest. High levels of IMAT have a strong association with metabolic and muscular dysfunction (16,33) and increase ~10% to 18% per year in mobility-limited older adults (20,34). In overweight adults, ectopic fat depots have been shown to be amenable through lifestyle-related interventions (18). In nonmobility limited older adults, high-intensity resistance training has been shown to improve levels of IMAT (21). However, the effects of moderate intensity physical activity on intermuscular fat and muscle density in mobility-limited older adults have been largely unexplored. Moreover, this is the first study to examine the effects of nutritional supplementation in combination with physical activity on thigh composition in older adults.

Only one previous trial has examined the effects of structured physical activity on thigh composition in a similar cohort (20). In this trial, physical activity was shown to prevent increases in intermuscular fat and declines in muscle density, when compared to control (20). The present study adds novelty to this work by demonstrating structured physical activity alone can lead to a decline in intermuscular fat. Further, the addition of a high protein, high vitamin D supplement elicits improvements in muscle density and greater declines in intermuscular fat than physical activity alone. This study also demonstrates improvements in muscle composition can be achieved without substantial weight loss. This finding is clinically relevant for this population as declines in muscle mass, which accompany weight loss, can lead to negative outcomes for older adults with low levels of muscle mass (35).

Interestingly, while gains in intermuscular fat and greater intramyocellular lipid content are associated with reduced strength and slower myofiber contraction kinetics (36,37), greater improvements in these lipid depots seen with nutritional supplementation were not concomitant with further improvements in muscle quality or mobility status (23). Although supplementation improved a pathological feature of metabolic and muscular dysfunction (38), the magnitude of change may not have been great enough to influence distal outcomes. The authors speculate that the time course of the intervention may have been too brief to capture therapeutic effects on strength and mobility-related outcomes. To what degree improvements in muscle composition influence clinically meaningful outcomes in this population remains to be established.

The direct mechanism leading to greater improvements in mid-thigh composition through nutritional supplementation is not known. However, vitamin D supplementation in older adults has been shown to influence serum myostatin and IGF-1 expression, both strong regulators of muscle mass and adipogenesis (11,39,40). Therefore, it is possible that addressing vitamin D deficiency in this population leads to a physiological milieu which promotes myogenesis and suppresses adipogenesis. However, a limitation to this study is that the multinutrient composition of the supplement makes it very difficult to definitively parse out which nutrient, or combination of nutrients, lead to the noted adaptations. Further, the nutritional supplement and placebo were not isocaloric.

The present study has many strengths including a robust sample, minimal lost to follow-up, and relatively long intervention period. However, some limitations include a lack of detailed information about usual dietary intake particularly with reference to protein. In addition, we did not specifically recruit participants with low or marginal nutritional status and this may have ultimately affected the robustness of our findings.

In conclusion, this trial supports the use of structured physical activity as a method for counteracting age-related muscular impairment. This study adds novelty to previous findings by demonstrating the additional benefits of a high protein, high vitamin D nutritional supplement on thigh composition. These results support the use of multimodal interventional strategies in older adults and warrant further investigation into the synergistic effects of exercise and nutritional supplementation which translate into positive outcomes in this population.

## Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.


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R.A.F. and T.C. designed research; R.A.F., D.R.K., A.K., A.B., M.L., and T.G. conducted research; T.T. and H.Z. analyzed data and performed statistical analysis; D.A.E. wrote the paper; D.A.E. and R.A.F. had primary responsibility for final content. All authors read and approved the final manuscript.


## Conflicts of Interest

R.A.F., T.T., and T.G. received grant support from Nestle' to support the trial. R.A.F. has received honoraria from Nestle'. The funders had no role in the conduct of the study.

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