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**Vitamin D intake, serum 25-hydroxyvitamin D status and response to moderate vitamin D<sub>3</sub> supplementation: a randomised controlled trial in East African and Finnish women**

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## Abstract

Insufficient vitamin D status (serum 25-hydroxyvitamin D (S-25(OH)D) < 50 nmol/l) is common among immigrants living at the northern latitudes. We investigated ethnic differences in response of S-25(OH)D to vitamin D<sub>3</sub> supplementation, through a 5-month randomised controlled trial, in East African and Finnish women in Southern Finland (60°N) in December 2014-May 2015. Vitamin D intakes (dietary and supplemental) were also examined. Altogether 191 subjects were screened and 147 women (East Africans n=72, Finns n=75) aged 21-64 years were randomised to receive placebo or 10 or 20 µg vitamin D<sub>3</sub>/d. S-25(OH)D concentrations were assessed by liquid chromatography-tandem mass spectrometry. At screening, 56% of East Africans and 9% of Finns had S-25(OH)D < 50 nmol/l. Total vitamin D intake was higher in East Africans than in Finns (24.2 [SD=14.3] vs. 15.2 [SD=13.4] µg/d, *p*<0.001). Baseline mean S-25(OH)D concentrations were higher in Finns (60.5 [SD=16.3] nmol/l) than in East Africans (51.5 [SD=15.4] nmol/l) (*p*=0.001). In repeated-measures analysis of covariance (adjusted for baseline S-25(OH)D), mean S-25(OH)D increased by 8.5 nmol/l and 10.0 nmol/l with a 10 µg dose and by 10.7 nmol/l and 17.1 nmol/l with a 20 µg dose for Finns and East Africans, respectively (*p*>0.05 for differences between ethnic groups). In conclusion, high prevalence of vitamin D insufficiency existed among East African women living in Finland, despite higher vitamin D intake than their Finnish peers. Moderate vitamin D<sub>3</sub> supplementation was effective in increasing S-25(OH)D in both groups of women, and no ethnic differences existed in the response to supplementation.

**Abbreviations:** 25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; EU, European Union; FFQ, food frequency questionnaire; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PTH, parathyroid hormone; SD, standard deviation; IOM, Institute of Medicine.

## Introduction

Serum 25-hydroxyvitamin D (S-25(OH)D) concentration is the most useful marker of vitamin D status<sup>(1)</sup>. Sufficient vitamin D status (S-25(OH)D > 50 nmol/l) is essential for bone health, especially in the prevention of secondary hyperparathyroidism, which causes osteoporosis and fractures, and in reducing risk of falls<sup>(1,2)</sup>. Vitamin D insufficiency (S-25(OH)D < 50 nmol/l), which has also been associated with risk for many types of cancer and other chronic diseases<sup>(2,3)</sup>, is a public health problem, affecting populations living at northern latitudes, especially during winter<sup>(4,5)</sup>. Nevertheless, the situation is not always the same between indigenous populations and immigrants. Vitamin D status in the majority of the native populations seems to be more satisfactory than among immigrants in the Nordic countries<sup>(6,7)</sup>.

Diet, namely fatty fish, fortified dairy products, fortified fat spreads and cod liver oil, and vitamin D supplements remain the main sources of vitamin D for the northern populations during the winter months, when sun-induced vitamin D synthesis in the skin is limited<sup>(7-10)</sup>. Unlike the indigenous populations, infrequent consumption of fatty fish and use of vitamin D supplements has been reported among immigrants of non-Western origin living in the Nordic countries<sup>(7,11)</sup>. Hence, in contrast to the case with indigenous populations, nutritional factors may contribute to the high prevalence of vitamin D deficiency observed among immigrants in the Nordic countries, especially among women<sup>(11)</sup>.

In particular, higher risk of vitamin D deficiency (S-25(OH)D <30 nmol/l) among immigrants of African and Asian background residing in northern countries has been reported in several studies<sup>(6,8,12,13)</sup>. Studies on skin colour and vitamin D synthesis have observed lower vitamin D status in individuals with dark skin than in those with lighter skin; skin pigmentation (melanin) interferes with vitamin D synthesis from ultraviolet B (UVB) exposure<sup>(14,15)</sup>. Wearing concealing clothing also contributes to an increased risk of vitamin D deficiency<sup>(4,7)</sup>. In Finland, high prevalence of S-25(OH)D < 30 nmol/l and S-25(OH)D < 50 nmol/l were observed in two recent studies of Somali<sup>(5,12)</sup> and Kurdish immigrants<sup>(5)</sup>. In contrast to the immigrants, sufficient vitamin D status was reported for the majority of Finnish adults in 2012<sup>(10)</sup>. Insufficient S-25(OH)D concentrations have also been observed in other studies examining immigrants of East African<sup>(16-18)</sup> and other ethnic origin<sup>(8,16,19)</sup> in the Nordic countries.

Despite these disparities in vitamin D status between the dark-skinned and fair-skinned populations, similar vitamin D recommendations based on studies among Caucasian populations are currently followed among both groups in the United States and in the Nordic countries<sup>(1,2)</sup>. However, there may be differences in vitamin D requirement and metabolism between the different population groups<sup>(20)</sup>. Concerns about the vitamin D status and requirements of dark-skinned immigrants residing in the Western world, the impact of ethnicity and the need for dose-response studies were highlighted in the Institute of Medicine (IOM) Dietary Reference Intakes report<sup>(1)</sup>.

Hence, the primary objective of this 5-month randomised controlled trial (RCT) was to investigate ethnic differences in the response of S-25(OH)D to vitamin D<sub>3</sub> supplementation over an extended winter period in women of East African and Finnish (Caucasian) descent. We also examined ethnic differences in vitamin D status with regard to S-25(OH)D concentrations and vitamin D intake from the diet and supplements in these two groups of women.

## **Methods**

### ***Study design and subject population***

This intervention study was implemented within the European Union (EU)-funded research project “Food-based solutions for optimal vitamin D nutrition and health through the life cycle” (ODIN; FP7-613977-ODIN; [www.odin-vitd.eu](http://www.odin-vitd.eu)). Specifically, the study was part of ODIN’s Work Package 6 with the overall objective of delivering the proof of efficacy and safety of food-based solutions to prevent vitamin D deficiency by focusing on EU-resident adults who are most at risk of vitamin D deficiency due to skin colour, sun exposure practices or dietary habits.

The study was a 5-month, randomised, placebo-controlled, dose-response (0, 10, 20 µg/d vitamin D<sub>3</sub>), trial conducted in December 2014 to May 2015. The study was tagged Marwo-D (the word was coined from the Somali word “Marwada” which means lady, and the letter D, which stands for vitamin D). The participants were recruited from the Helsinki metropolitan area (latitude 60°N). The participants of East African descent were recruited from the register of subjects who were shortlisted for participation in the cross-sectional Migrant Health and Wellbeing Study (Maamu), a population-based health interview and examination survey among immigrants in Finland<sup>(21)</sup>, and also from mosques and meeting places (outside Maamu sample). The indigenous Finnish participants were recruited from the Health 2011 survey, a study carried out among the Finnish mainland population<sup>(22,23)</sup>, and through advertisements in social media and on the Viikki campus area of the University of Helsinki. Invitation letters were sent to participants from the Maamu and Health 2011 samples, and they were subsequently contacted by telephone. All women of East African descent were first generation immigrants with Somali origin, dark-skinned and wore traditional clothing.

A total of 191 subjects were screened for eligibility. The inclusion criteria were female sex, Somali or Finnish origin, body mass index (BMI)  $\leq 40$  kg/m<sup>2</sup> and S-25(OH)D concentration  $>30$  but  $<100$  nmol/l. The inclusion criteria for S-25(OH)D concentration was based on ethical viewpoint (not to include deficient subjects, who need supplementation) and ability to evaluate the response to supplementation (which may not be obvious among participants with higher S-25(OH)D concentrations). Exclusion criteria included pregnancy or breastfeeding during the study, a vacation in a sunny destination prior to or during the study, use of a tanning bed prior to or during the study and medication or illnesses that interfere with vitamin D metabolism. Medical history, S-25(OH)D concentration and other inclusion and exclusion criteria were assessed during screening in October/November 2014. Subjects who did not meet the inclusion criteria due to S-25(OH)D concentration  $<30$  nmol/l (i.e. vitamin D-deficient) received information on dietary and supplemental sources of vitamin D, and they were advised to contact their health care services for further medical actions. In addition, they were given either 10 or 20 µg vitamin D<sub>3</sub> supplements for daily use, depending on the severity of deficiency. Altogether 147 women (77% of those screened);

72 (49%) of East African descent and 75 (51%) of Finnish descent, aged 21-64 years, met the inclusion criteria and were studied at the Calcium Research Unit of University of Helsinki, Finland. Participants' recruitment and randomisation are presented in Fig. 1 (Consort diagram).

The study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District. Written informed consent was obtained from all subjects. The consent form and the participant information sheet were provided in Somali and Finnish languages. The study was registered as a clinical trial on ClinicalTrials.gov ([www.ClinicalTrials.gov](http://www.ClinicalTrials.gov); NCT02212223).

### ***Randomisation and intervention***

The East African and Finnish women who met the inclusion criteria were randomised into three supplementation groups: placebo or 10 µg or 20 µg vitamin D<sub>3</sub>/d (Fig. 1). Participants were evenly randomised into intervention groups, for group similarities with respect to the distribution of S-25(OH)D, BMI, age, habitual vitamin D intake from supplements at screening and ethnicity. Altogether 125 subjects (85% of those randomised) completed the study and 22 subjects (15%) discontinued after randomisation. The reasons for discontinuation were as follows: withdrawal (n=8), lost to follow-up (n=8), ineligible due to pregnancy (n=6). Supplements containing 10 µg or 20 µg vitamin D<sub>3</sub> per tablet and identical placebo tablets containing 0 µg were provided by Oy Verman Ab (Kerava, Finland). Supplements and placebo tablets were kept in similar jars identifiable only by the subjects' ID numbers. Each 10 µg vitamin D<sub>3</sub> tablet coincides with the Finnish national recommendation for daily vitamin D intake for the studied age group, while each 20 µg tablet is twice the Finnish national recommendation for daily vitamin D intake<sup>(24)</sup>.

Each participant received a jar containing placebo or vitamin D<sub>3</sub> tablets at the baseline visit (in December) and subsequently at the midpoint visit (in February/March). They were advised to take one tablet daily and were given research diaries to keep a record of their study tablet use and occurrence of any side effects during the intervention period. At the midpoint and endpoint (in April/May) visits, compliance was calculated by counting the remaining tablets in the returned jars. The participants' research diaries were evaluated at each visit during the intervention.

Participants were not restricted from taking their personal vitamin D-containing supplements; those who used vitamin D supplements before the study were advised to continue in the same manner throughout the intervention period in order to avoid changes in their habitual vitamin D intake. Participants who had no previous personal vitamin D supplementation but wished to start were

allowed to use supplements at doses  $\leq 10$   $\mu\text{g}$  vitamin D/d. Possible changes in the use of personal vitamin D-containing supplements were monitored during the intervention period.

### ***Background and dietary data collection***

Background data was collected from all participants through a detailed questionnaire either delivered via interview (women of East African descent) or self-administered (women of Finnish descent). Vitamin D supplementation practices were assessed based on how often vitamin D-containing supplements were used, dosage and trademark; questions on general health included any experience of health problem and specific medication; habitual sunshine exposure was measured by type of clothing worn outdoors in summer, and working hours and leisure time spent outdoors during summer. Weight and height were measured at screening and BMI was calculated as weight (kg) / height (m)<sup>2</sup>.

Habitual vitamin D intake was assessed in all participants, based on a validated semi-quantitative interview-administered food frequency questionnaire (FFQ)<sup>(25)</sup>, at the baseline and endpoint of the intervention. The FFQ used in this study covered 9 food groups, comprising altogether 46 food items, considered important sources of vitamin D (Table 1). Vitamin D intake during the previous month was assessed with closed questions on consumption frequencies (daily, weekly, monthly, less often or not at all) and portion sizes (e.g. 1 glass or 1 piece). Open questions were asked with regard to use of fat (such as spread on bread, in cooking and baking). Information on brand name of food products was also included in the FFQ.

Interviews with women of Finnish descent were conducted in Finnish. Some of the women of East African descent were interviewed entirely in the Finnish language, while others were interviewed with translation by Somali-speaking research assistants, when necessary. A picture booklet of products fortified with vitamin D was used to help participants identify consumed products. Pictures showing portion sizes were also used when needed. The questionnaire was piloted in a small East African population (n=5) prior to its administration in the study.

The dietary vitamin D intakes were calculated based on the FFQ consumption data for vitamin D fortified fluid milk products and fat spreads, fish and other sources (such as milk-based foods, main courses, mushrooms and other vitamin D-fortified products, namely cheese, bread, juice and mineral water), using the Finnish national food composition database, Fineli<sup>®</sup>, which was developed and is continuously updated by the Nutrition Unit of the Finnish National Institution of Health and Welfare ([www.fineli.fi](http://www.fineli.fi)).

### ***Blood sample collection***

Fasting blood samples were collected at screening, baseline, midpoint and endpoint visits, between 6:45 a.m. and 12:30 p.m. After serum separation, samples were stored frozen at  $-70^{\circ}\text{C}$  until analysis. Total S-25(OH)D concentration was assessed from the serum samples (i.e. at screening, baseline, midpoint and endpoint) by the Cork Centre for Vitamin D and Nutrition Research at the University College Cork, Ireland, using liquid chromatography-tandem mass spectrometry (LC-MS/MS) which is the central analytical platform for the ODIN project. The LC-MS/MS method measures S-25(OH)D<sub>2</sub> and S-25(OH)D<sub>3</sub> separately, and total S-25(OH)D concentrations were calculated as the sum of these values. The inter-assay and intra-assay CVs for the analyses were <5% and <6%, respectively, for both metabolites<sup>(26)</sup>. The quality and accuracy of S-25(OH)D analysis by the LC-MS/MS in the laboratory is guaranteed on an ongoing basis by participation in the Vitamin D External Quality Assessment Scheme (DEQAS) (Charing Cross Hospital)<sup>(26)</sup>. Moreover, the Cork Centre for Vitamin D and Nutrition Research's method is certified under the Centers for Disease Control and Prevention Vitamin D Standardization Certification Program ([http://www.cdc.gov/labstandards/pdf/hs/CDC\\_Certified\\_Vitamin\\_D\\_Procedures.pdf](http://www.cdc.gov/labstandards/pdf/hs/CDC_Certified_Vitamin_D_Procedures.pdf)). In line with IOM S-25(OH)D thresholds for an adult population, we defined vitamin D status of S-25(OH)D < 30 nmol/l as deficient; S-25(OH)D of 40 nmol/l as concentrations that cover the requirements of 50% of the population; S-25(OH)D 30 - < 50 nmol/l as insufficient; S-25(OH)D  $\geq$  50 nmol/l as sufficient; sustained S-25(OH)D concentration > 125 nmol/l raised concerns about possible adverse effects<sup>(1)</sup>. For study purposes, we also defined S-25(OH)D concentrations in the range 75 -125 nmol/l as higher concentrations.

Serum parathyroid hormone (S-PTH) concentrations were analysed by an immunoluminescence-based method using Immulite1000 (Siemens Healthcare Diagnostics) at the Department of Food and Nutrition, University of Helsinki, with inter-assay and intra-assay CVs of <8.0% and <5.5%, respectively. Serum calcium (S-Ca), albumin and phosphorus (S-Pi) concentrations were assessed with a photometric method using Konelab20 automatic analyser (Thermo Clinical Labsystems Oy) at the Department of Food and Nutrition, University of Helsinki. The inter-assay and intra-assay CVs for S-Ca and S-Pi analyses were <4.6% and <4.6%, respectively. S-Ca results were used as albumin-corrected.

### ***Statistical analysis***

A power calculation based on the S-25(OH)D concentrations was performed to estimate the number of subjects needed. On the basis of the distribution of wintertime serum 25(OH)D data from our previous study of white adult Finnish women<sup>(27)</sup>, we calculated that 34 volunteers per group should be recruited, with 90% power to detect a minimum of a 10 nmol/l increase in serum 25(OH)D



between groups, within an ethnic group, at  $\alpha = 0.5$ . However, this number was increased to 40 for each dose group (placebo, 10  $\mu\text{g/d}$  and 20  $\mu\text{g/d}$  in each ethnic group) to account for possible dropouts. A total of 240 women (120 in each ethnic group) were aimed to be enrolled but the targeted sample size could not be reached due the seasonal timeframe (i.e. wintertime) of the study. Hence, we could not extend the recruitment period for more participants. We assumed the distribution of wintertime serum 25(OH)D would be similar for non-white adult Finnish women and used similar numbers per group.

Normality of the distribution of variables was tested with Kolmogorov-Smirnov test. Analysis of variance (ANOVA) was used to assess differences in normally distributed variables in intervention groups within both ethnic groups, while differences in non-normally distributed variables were evaluated with non-parametric test (Kruskal Wallis). Comparison of variables between the two ethnic groups was performed with t-test (normally distributed variables) and non-parametric Mann-Whitney U test (non-normally distributed variables). Repeated-measures analysis of covariance (ANCOVA) was used to evaluate the effect of supplementation on S-25(OH)D, S-PTH, S-Ca and S-Pi in the two ethnic groups. In ANCOVA, the baseline S-25(OH)D, S-PTH, S-Ca or S-Pi concentration was used as a covariate. Comparisons between intervention groups were carried out with contrasts. Results are presented as mean values and standard deviations (SD), and in figures as means with standard errors. All results were considered statistically significant at  $p < 0.05$ . IBM Statistical Package for the Social Sciences Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis.

In the analysis focusing on the effects of intervention, three East African subjects randomised to the group that received 20  $\mu\text{g/d}$  (initial  $n=21$ ) were moved to the placebo group (initial  $n=25$ ) because one of the three participants stopped the supplementation after four days, the other two participants did not take the supplement at all. Final analysis included only the participants who completed the intervention and had  $\geq 80\%$  compliance rate with study supplementation in each group. Nevertheless, three participants were excluded from the analysis for the following reasons: pregnancy ( $n=1$ ), kidney dysfunction ( $n=1$ ) and sunburn ( $n=1$ ). Subjects on medications due to hypothyroidism ( $n=5$ ) and type 2 diabetes ( $n=5$ ) were included in the analysis because their exclusion had no significant effect on the results. Altogether, data from 116 participants were analysed. Additional analyses were performed for the evaluation of vitamin D status in the two ethnic groups with regard to S-25(OH)D concentrations in all screened subjects ( $n=191$ ) and vitamin D intake from the diet and supplements in all randomised subjects ( $n=147$ ).

## Results

### ***Serum 25-hydroxyvitamin D at screening screened***

We screened altogether 191 subjects (East African women n=104, Finnish women n=87). Based on the IOM thresholds for S-25(OH)D concentrations, 17% (n=18) and 39% (n=40) of the screened East African women were deficient (S-25(OH)D < 30 nmol/l) or had insufficient status (S-25(OH)D 30 - < 50 nmol/l), respectively<sup>(1)</sup>. There was no vitamin D deficiency observed in Finnish women and the proportion of participants who had insufficient status (9%) (n=8) was lower than in East African women. Sufficient vitamin D status (S-25(OH)D ≥ 50 nmol/l) was observed in 44% (n=46) of East African women and in 91% (n=79) of Finnish women. Higher concentrations (S-25(OH)D 75 - 125 nmol/l) were seen in 7% (n=7) of East African women and 33% (n=29) of Finnish women. S-25(OH)D concentrations above 125 nmol/l were observed in 5% (n=4) of Finnish women (Table 2).

### ***Characteristics of the participants in the intervention***

Among the 147 randomised participants (East African women n=72, Finnish women n=75), the mean compliance with study supplementation in women of East African descent was 89% (n=54) and in women of Finnish descent 98% (n=71). The baseline mean S-25(OH)D concentrations were higher in Finnish women (mean: 60.5 [SD=16.3] nmol/l) than in East African women (mean: 51.5 [SD=15.4] nmol/l) ( $p=0.001$ ), (data not shown). The characteristics of the 116 participants included in the final analyses are shown in Table 3. The intervention groups in each ethnicity did not differ from one another with regard to any background data. Nevertheless, women of East African descent differ from Finnish women in all characteristics, except for S-Pi concentrations.

### ***Baseline vitamin D intake and sources of vitamin D***

Total vitamin D intake at baseline as well as that from diet and supplements (where relevant) separately, and stratified by supplement non-use and voluntary supplement use (participants who used their personal supplements in addition to study supplements) are given in Table 4. Higher mean habitual vitamin D intakes from the diet (11.2 [SD=5.8] vs. 8.4 [SD=4.1] µg/d,  $p=0.003$ ) and supplements (13.0 [SD=5.8] vs. 6.9 [SD=4.1] µg/d,  $p<0.001$ ) were observed in East African women (n=72) than in Finnish participants (n=75) (Table 4). The proportions of voluntary supplement users in East African women and Finnish women were 88% (n=63) and 47% (n=35), respectively. Meanwhile, similar mean intakes from supplements were observed among the voluntary vitamin D supplement users in both ethnic groups (East African women=14.5 [SD=11.4] µg; Finnish women=14.7 [SD=15.1] µg,  $p>0.05$ ) (Table 4). Participants using personal vitamin D supplements demonstrated higher total mean vitamin D intake than those not using personal supplements (East

African women=26.0 [SD=13.9] vs. 8.7 [SD=7.4]  $\mu\text{g}$ ,  $p=0.002$ ; Finnish women=23.9 [SD=15.1] vs. 7.6 [SD=4.1]  $\mu\text{g}$ ,  $p<0.001$ ) (Table 4). The proportion of participants attaining the daily recommended vitamin D intake of 10  $\mu\text{g}$ <sup>(2,24)</sup> from diet and supplements was higher in East African women (83%) (n=60) than in Finnish women (55%) (n=41) ( $p=0.003$ ), (data not shown).

The contributory food sources to the mean daily intake of vitamin D for both East African women and Finnish women are presented in Fig. 2. The major source of vitamin D for the two groups of women was fortified fluid milk products, with higher intake in East African women (East African women=5.2 [SD=4.0]  $\mu\text{g}$ ; Finnish women=3.4 [SD=3.0]  $\mu\text{g}$ ,  $p=0.003$ ). Similar vitamin D intake from fortified fat spread and fish was observed in both groups ( $p>0.05$ ).

### ***Effect of vitamin D supplementation on S-25(OH)D, S-PTH, S-Ca and S-Pi***

After the 5-month intervention, the effect of vitamin D<sub>3</sub> supplementation on S-25(OH)D among the 116 participants included in the analysis are shown in Fig. 3 (repeated-measures ANCOVA, adjusted for baseline S-25(OH)D concentration); compared with placebo, vitamin D supplementation with both 10  $\mu\text{g}$  and 20  $\mu\text{g}$  doses significantly increased S-25(OH)D concentration in both ethnic groups. No significant differences were seen between 10  $\mu\text{g}$  and 20  $\mu\text{g}$  dosages ( $p>0.05$ ) in either of the two ethnic groups. We observed no differences in the results when we excluded the three East African subjects that were moved from 20  $\mu\text{g}$  to the placebo group. The results did not change after adjustment for personal supplement use, dietary vitamin D intake and BMI. Using a regression model, there was no significant difference in intake-serum 25(OH)D response between women of East African descent and those of Finnish descent when baseline serum S-25OHD concentration was included as a covariate ( $p>0.3$ ; data not shown). The mean changes during the study period in the 10  $\mu\text{g}$  D<sub>3</sub> supplement groups for Finnish women and East African women were +8.5 nmol/l (+14.1%) and +10.0 nmol/l (+19.2%), respectively, and in the 20  $\mu\text{g}$  D<sub>3</sub> supplement groups +10.7 nmol/l (+17.7%) and +17.1 nmol/l (+32.7%), respectively. The mean changes in placebo groups for Finnish women and East African women were -7.8 nmol/l (-13.0%) and -2.3 nmol/l (-4.4%), respectively. Between the two groups of women, no significant differences were observed in response to vitamin D<sub>3</sub> supplementation ( $p>0.05$ ) (Fig. 3). No significant effect of vitamin D<sub>3</sub> supplementation on S-PTH, S-Ca or S-Pi was seen in either East African or Finnish women ( $p>0.05$ ) (repeated-measures ANCOVA, adjusted for baseline S-PTH/S-Ca/S-Pi, data not shown).

## **Discussion**

This 5-month intervention was the first randomised controlled vitamin D dose-response study in East African and Caucasian women starting during the winter months, without natural UVB irradiation. The study demonstrated that supplementation with 10 µg and 20 µg vitamin D<sub>3</sub> was effective in increasing S-25(OH)D in both East African and Finnish women, while a significant decrease in S-25(OH)D concentrations occurred with placebo in both ethnic groups. No ethnic differences in S-25(OH)D response to vitamin D<sub>3</sub> supplementation between the two ethnic groups were present.

To date, only a few dose-response vitamin D supplementation studies have been conducted in ethnically diverse populations<sup>(28-30)</sup>. Our study found no ethnic differences in S-25(OH)D response to vitamin D<sub>3</sub> supplementation between East African and Finnish women, consistent with earlier findings that the effect of dose on S-25(OH)D is independent of race<sup>(28,29)</sup>. The two groups of women responded to vitamin D<sub>3</sub> supplementation in the same way.

Although, our results did not change after adjustment for BMI, the higher BMI found in East African women might have contributed to their lower mean S-25(OH)D concentrations. Studies have shown an inverse association between BMI and S-25(OH)D concentrations<sup>(31-34)</sup> since large fat mass reduces the bioavailability of synthesized vitamin D deposited in the body fat compartment<sup>(4,35)</sup>. Negative effects of BMI  $\geq 25$  kg/m<sup>2</sup> on S-25(OH)D have been described among African Americans<sup>(32,36)</sup>. According to Drincic et al.<sup>(34)</sup>, lower S-25(OH)D concentration in obese individuals was attributed to volumetric dilution of ingested or cutaneous vitamin D in the large fat mass. On the other hand, inconsistent results on the effect of BMI on dose-response of S-25(OH)D to vitamin D supplementation have been reported in some studies<sup>(37,38)</sup>. For instance, Grønberg et al.<sup>(39)</sup> found no association between body fat and vitamin D status, and also that body fat had no effect on the response to vitamin D supplementation. Genetic factors may also influence S-25(OH)D circulation<sup>(40,41)</sup>. A probable link between genetic background and response to vitamin D supplementation or dietary vitamin D exists; for instance, polymorphisms of the vitamin D receptor, vitamin D-binding protein or other genetic determinates of S-25(OH)D have been reported<sup>(42,43)</sup>. A similar situation with women of African ancestry may exist in our study. Studies are needed to investigate association between genetic factors and S-25(OH)D among dark-skinned populations as the findings may be different from that among the Caucasian populations.

In the screening, less than half of the East African women had sufficient S-25(OH)D concentrations, while 9 of 10 of their Finnish peers reached the 50 nmol/l as suggested by the IOM<sup>(1)</sup> to cover the needs of 97.5% of the population with regard to bone health. Besides IOM recommendation, the Endocrine Society suggested S-25(OH)D concentrations above 75 nmol/l for both bone and non-skeletal functions<sup>(44)</sup>, and this was achieved by 38% of Finnish women but only

7% of East African women. Similarly to our study, lower S-25(OH)D concentrations were observed in Somali women (East Africans) than in Finnish women in an earlier study carried out in Finland<sup>(12)</sup>. In other countries besides Finland, high prevalence of vitamin D deficiency (S-25(OH)D <30 nmol/l) is commonly reported in Somali subjects (East Africans)<sup>(16-18)</sup>. On the contrary, vitamin D status in the general Finnish population has improved over the years and it is satisfactory<sup>(10,45)</sup>. The S-25(OH)D concentrations in this study is comparable to that of the general population. Nonetheless, variation in prevalence of vitamin D deficiency among the European populations has been described<sup>(5)</sup>. The inexistence of vitamin D deficiency and low insufficiency observed in Finnish women of our study may not represent other European populations, as higher vitamin D deficiencies have been reported<sup>(6,17)</sup>.

Effectiveness of 10 µg or 20 µg vitamin D<sub>3</sub> supplementation has been observed in studies involving participants with baseline mean S-25(OH)D concentrations above 50 nmol/l<sup>(46,47)</sup>. Our results were also consistent with previous RCTs among Pakistani immigrants<sup>(19)</sup> and Finnish women<sup>(48)</sup>, as we observed an increase in mean S-25(OH)D concentrations with 10 µg and 20 µg dosages of vitamin D<sub>3</sub> supplementation in both East African and Finnish women during the 5-month intervention. The mean S-25(OH)D concentrations decreased with placebo in both ethnic groups. The previous studies<sup>(19,48)</sup> reported greater increments, which means stronger response to vitamin D<sub>3</sub> supplementation, due to lower basal S-25(OH)D concentrations than in our study. Considering the effect of baseline S-25(OH)D concentration on response to supplementations, other RCTs<sup>(31,48,49)</sup> carried out, spanning over one year or less, among subjects with vitamin D insufficiency found an increase in the S-25(OH)D concentration above 50 nmol/l. For instance, in the studies by Gallagher et al., 10 µg of vitamin D<sub>3</sub> increased S-25(OH)D concentrations by an average of 32.5 nmol/l, while 20 µg dose sufficiently increased S-25(OH)D above 50 nmol/l in 98% and 97.5% of both Caucasian (baseline S-25(OH)D =39 nmol/l) and African American women (baseline S-25(OH)D =33 nmol/l), respectively<sup>(29,31)</sup>. These suggest that daily vitamin D supplementation at doses between 10 µg and 20 µg is probably adequate to maintain optimal S-25(OH)D concentrations without sunlight exposure during winter. Hence, doses above 20 µg may not have substantial additional benefits among persons with sufficient vitamin D concentrations.

Of interest, there was a substantial contribution of dietary sources to daily vitamin D intake, fortified fluid milk products being the major source of vitamin D for both groups of women, with higher intake in East African women. Though, lower consumption of vitamin D fortified milk was reported among immigrant women than the native Swedish reference group<sup>(6)</sup>, milk from camels, cattle or goats is one of the staple diets (also beverage) in Somalia and this may explain high milk consumption in our study<sup>(50-52)</sup>. Vitamin D intake from fortified fat spreads and fish were similar in

both groups. Similar frequent consumption of fortified milk products, as one of the main dietary sources of vitamin D, has been reported in the general Finnish population<sup>(10)</sup>. In both East African and Finnish women, there was similar dietary vitamin D intake among supplement users and non-users. The mean dietary vitamin D intake in both groups of women almost reached the 10 µg daily recommended intake of vitamin D<sup>(24)</sup>. However, use of supplements increased the total mean vitamin D intake above the daily recommendation among the supplement users in both ethnic groups. Unlike in previous studies<sup>(6,12)</sup>, the rate of supplement usage in our study was higher among the immigrant group. Nonetheless, higher vitamin D intake may not necessarily translate to higher S-25(OH)D concentration<sup>(12)</sup>. Despite higher dietary intake and personal supplementation observed in East African women, their mean S-25(OH)D concentrations were lower than in Finnish women. Our experience of higher vitamin D intake and lower S-25(OH)D among East African women reflects lower endogenous vitamin D synthesis from UVB radiation during summer. Such absence of vitamin D production in the skin emphasizes the relationship between the use of concealing clothes during summer and lower S-25(OH)D concentrations, especially among women<sup>(4,7)</sup>. According to Gallagher et al.<sup>(29)</sup>, absorption and metabolism of vitamin D in African American and Caucasian women are similar. Hence, the reported lower S-25(OH)D concentrations in dark-skinned individuals probably occur due to decreased formation of vitamin D in the skin<sup>(38)</sup>. Besides oral vitamin D intake, this result suggests the presence of other factors (such as BMI and genetic factors) affecting S-25(OH)D concentrations in these women.

Some factors emerged as limitations against generalisation of this study's findings. First, the baseline mean S-25(OH)D concentrations of the two groups of women included in the trial were quite sufficient (baseline mean S-25(OH)D > 50 nmol/l). This means that the results may be different in subjects with vitamin D deficiency. Second, use of personal vitamin D supplement was not restricted during the intervention and a high proportion of personal vitamin D supplementation was found in East African women. Third, the homogeneity of the participants with regard to sex (only women) limits the applicability of the results to men in the population. Fourth, although the FFQ used to assess the vitamin D intake of all subjects was piloted among East African women, it was only validated among Finnish women (Caucasians)<sup>(25)</sup>. Thus, complete vitamin D intake in East African women might have not been evaluated. In addition, measurement error of the FFQ, such as over-reporting, might have contributed to the higher vitamin D intakes among East African women. The reported high consumption of fortified fluid milk may be culturally related to perceived status of milk as important staple diet in their home country.

One of the strengths of this research lies in the study design (randomised, placebo-controlled), which allowed for an objective evaluation of the effects of vitamin D<sub>3</sub> doses on S-25(OH)D.

Evaluation of compliance with vitamin D supplementation in this study is considered a strength, and dietary vitamin D intakes that were assessed at two different points proved the reliability of our data. Compliance rates with study supplementation, blood sampling and questionnaires, including FFQ, in both ethnic groups of women were high. Use of the participants' preferred language, including translation (when necessary), during interviews enhance the quality of our data. Our first of its kind study provides up-to-date data on vitamin D intake and status in Finnish and East African women in Finland.

## **Conclusions**

Supplementation with moderate vitamin D<sub>3</sub> doses increased the S-25(OH)D concentrations in both East African and Finnish women during the 5-month intervention. Our study supports earlier findings that ethnicity has no effect on the response of S-25(OH)D to vitamin D<sub>3</sub> supplementation. Future studies should focus on identifying the factors, other than dietary, associated with the greatest risk of vitamin D insufficiency in dark-skinned populations.

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The authors declare no conflicts of interest.

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## Figure legends:

**Fig. 1.** Consort diagram. Details of the recruitment, randomisation and distribution of the participants in the Marwo-D study. EA = women of East African descent; FIN = women of Finnish descent; S-25OHD = serum 25-hydroxyvitamin D; BMI = body mass index

**Fig. 2.** Baseline daily vitamin D intake from dietary sources. Values are mean, vitamin D intakes calculated from baseline FFQ. ■ Fortified fluid milk products; ■ Fortified fat spreads; □ Fish; □ Others (milk-based foods, main courses, mushrooms, vitamin D-fortified cheese, bread, juice and mineral water)

**Fig. 3.** Response of serum 25-hydroxyvitamin D (S-25(OH)D) to vitamin D<sub>3</sub> supplementation in women of East African (a) and Finnish (b) descent (adjusted for baseline S-25(OH)D concentrations, repeated-measures ANCOVA). The time points are at 2.5-month intervals, representing mean values at each time point, error bars represent standard errors. Comparisons between intervention groups using contrasts: \*\* $p < 0.001$  and \* $p = 0.003$  for the differences compared to placebo;  $p = 0.105$  for East African women and  $p = 0.308$  for Finnish women for differences between 10 µg and 20 µg groups in both ethnic groups.

**Table 1.** Food groups in the FFQ

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Food groups
Milk and plant-based drinks (soy, oat, etc.)
Yoghurts, curdled milks and quarks
Cheeses
Milk-based foods
Main courses, meat dishes and egg
Mushrooms
Fishes
Fat as spread on bread, in cooking and baking
Vitamin fortified juices and mineral waters

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**Table 2.** Vitamin D status of subjects (n=191) at screening according to S-25OHD concentration thresholds (numbers and percentages).

S-25OHD (nmol/l) categories	East African women (n=104)		Finnish women (n=87)	
	N	%	N	%
<30	18	17.3	0	-
30 - <40	21	20.2	3	3.4
40 - <50	19	18.3	5	5.8
50 - <75	39	37.5	46	52.9
75 - <125	7	6.7	29	33.3
≥125	0	-	4	4.6

**Table 3.** Characteristics of the participants (n=116) stratified by intervention group and ethnicity

	East African women				<i>p</i> value (between intervention groups)	Finnish women				<i>p</i> value (between intervention groups)	<i>p</i> value (between all East African & Finnish women)
	Dose 0 µg n=22	Dose 10 µg n=15	Dose 20 µg n=10	All n=47		Dose 0 µg n=23	Dose 10 µg n=23	Dose 20 µg n=23	All n=69		
Age (years)†	42.6 (9.0)	40.5 (7.1)	39.3 (7.0)	41.2 (8.0)	0.523	32.7 (7.6)	32.8 (8.4)	32.7 (8.4)	32.7 (8.0)	0.994	<b>0.000</b>
Height (cm)	163.4 (6.1)	162.0 (5.2)	164.0 (3.8)	163.1 (5.3)	0.619	164.5 (7.0)	165.8 (5.7)	168.2 (5.7)	166.2 (6.2)	0.124	<b>0.006</b>
Weight (kg)†	78.0 (15.2)	77.9 (9.2)	79.1 (15.3)	78.2 (13.3)	0.963	62.1 (9.5)	68.4 (14.9)	66.4 (10.5)	65.7 (12.0)	0.262	<b>0.000</b>
BMI (kg/m <sup>2</sup> )†	29.3 (5.8)	29.7 (3.2)	29.3 (5.1)	29.4 (4.8)	0.969	23.0 (3.5)	24.8 (4.7)	23.5 (3.9)	23.8 (4.0)	0.447	<b>0.000</b>
Dietary vitamin D intake (µg/d)†	11.2 (5.2)	12.0 (5.8)	10.5 (4.2)	11.3 (5.1)	0.931	7.7 (3.2)	8.6 (5.1)	9.0 (3.9)	8.4 (4.1)	0.469	<b>0.002</b>
Vitamin D intake from personal supplement (µg/d)†	10.0 (5.6)	12.5 (14.8)	12.2 (10.1)	11.2 (10.1)	0.891	5.3 (10.5)	7.0 (9.8)	4.8 (7.6)	5.7 (9.3)	0.550	<b>0.000</b>
Total vitamin D intake from diet and personal supplement (µg/d)†	21.1 (8.4)	24.5 (17.8)	22.6 (11.8)	22.5 (12.6)	0.922	13.0 (12.3)	15.6 (10.6)	13.8 (8.5)	14.1 (10.5)	0.408	<b>0.000</b>
Baseline serum 25OHD (nmol/l)	52.6 (12.9)	51.6 (13.9)	52.2 (17.8)	52.2 (14.0)	0.979	59.7 (16.5)	60.8 (17.1)	61.0 (16.8)	60.5 (16.6)	0.962	<b>0.006</b>
Baseline serum PTH (pg/ml)†*	47.0 (19.0)	50.2 (32.8)	35.2 (24.5)	45.2 (24.7)	0.124	32.4 (16.8)	29.5 (15.3)	40.6 (17.8)	34.1 (17.0)	0.086	<b>0.021</b>
Baseline albumin-corrected calcium (mmol/l)	2.54 (0.10)	2.53 (0.07)	2.56 (0.11)	2.54 (0.09)	0.565	2.51 (0.11)	2.49 (0.71)	2.48 (0.07)	2.49 (0.09)	0.546	<b>0.002</b>
Baseline serum phosphorus (mmol/l)	1.31 (0.12)	1.23 (0.18)	1.25 (0.11)	1.27 (0.14)	0.233	1.28 (0.20)	1.30 (0.16)	1.32 (0.15)	1.30 (0.17)	0.750	0.291

Values are mean (SD), vitamin D intakes calculated from FFQ (mean of baseline and endpoint). BMI = body mass index. 25OHD = 25-hydroxyvitamin D

Significant differences between East African and Finnish women are presented in bold. *P* values <0.05 from † non-parametric tests, ANOVA and T-tests

\* n=112 for Serum PTH analysis



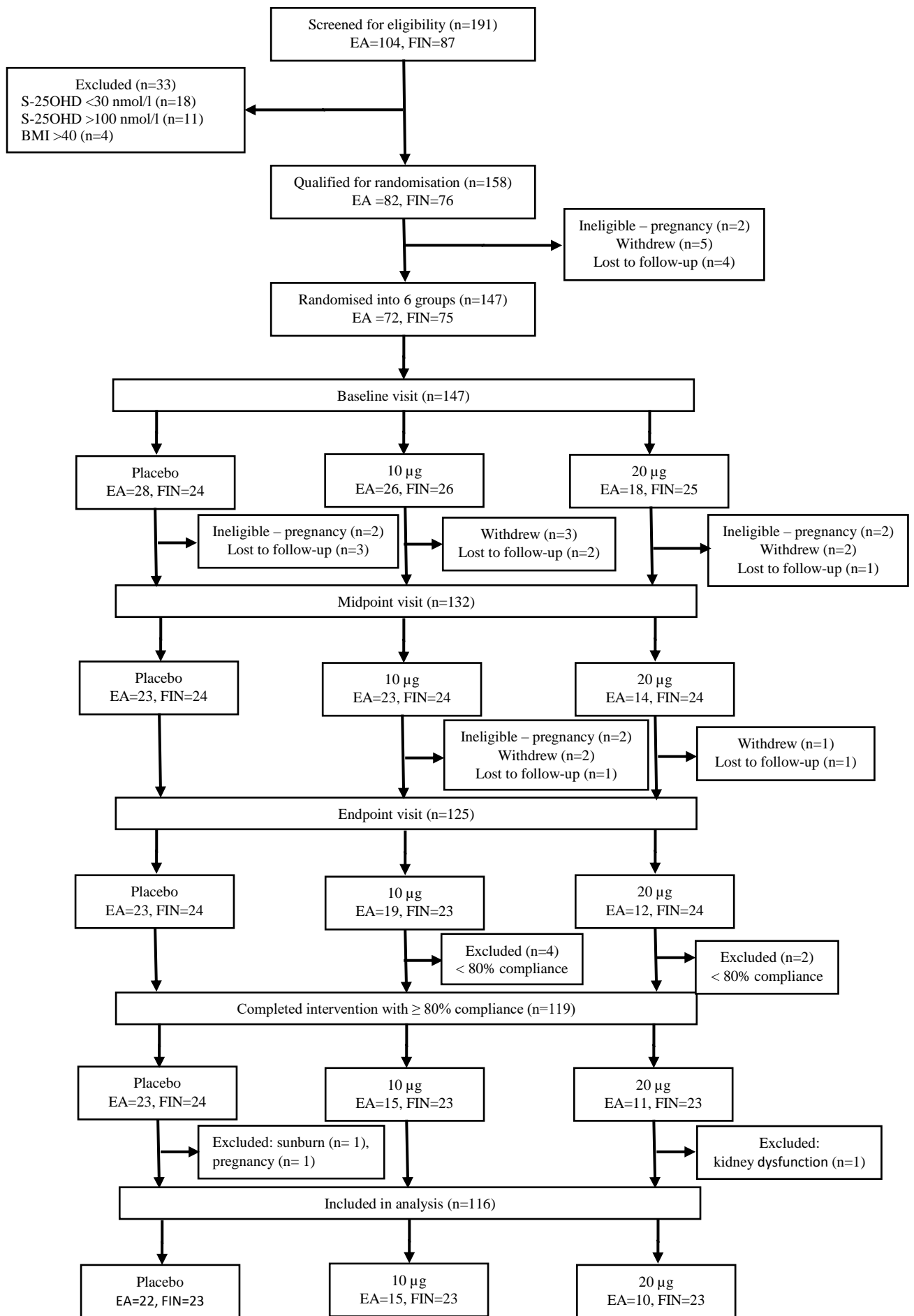
**Table 4.** Daily vitamin D intake ( $\mu\text{g}/\text{d}$ ) from the diet and supplements at baseline (n=147).

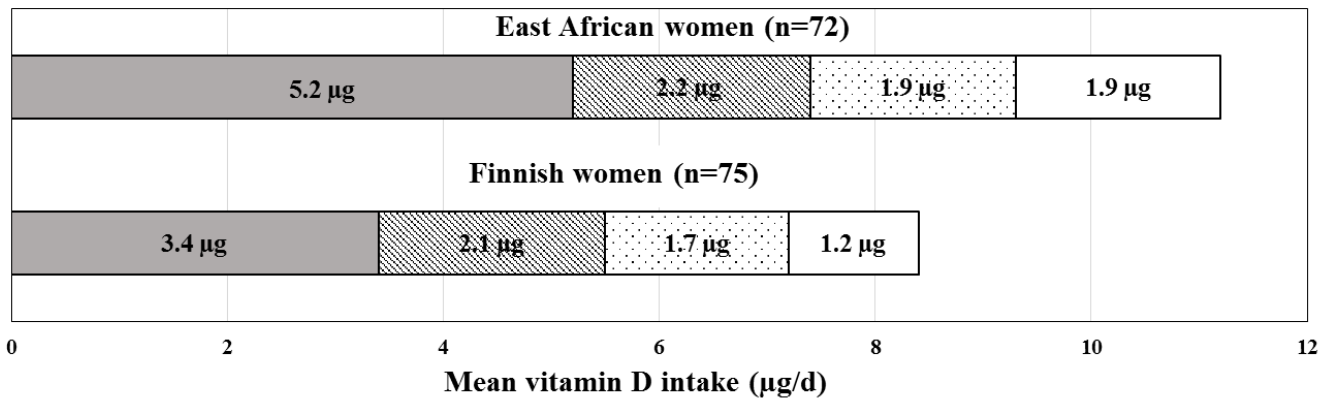
Vitamin D intake ( $\mu\text{g}/\text{d}$ )	East African women (n=72)				Finnish women (n=75)				
	All Mean (SD)	Supplement non-users (n=9) Mean (SD)	Supplement users (n=63) Mean (SD)	<i>P</i> value (between Supplement non-users and users)	All Mean (SD)	Supplement non-users (n=40) Mean (SD)	Supplement users (n=35) Mean (SD)	<i>P</i> value (between Supplement non-users and users)	<i>P</i> value (between all East African & Finnish women)
From diet	11.2 (5.8)	8.7 (7.4)	11.6 (5.5)	0.082	8.4 (4.1)	7.6 (4.1)	9.2 (4.0)	0.063	<b>0.003</b>
From supplement	13.0 (11.6)	-	14.5 (11.4)	<b>0.000</b>	6.9 (12.6)	-	14.7 (15.1)	<b>0.000</b>	<b>0.000</b>
Total intake from diet and supplement	24.2 (14.3)	8.7 (7.4)	26.0 (13.9)	<b>0.002</b>	15.2 (13.4)	7.6 (4.1)	23.9 (15.1)	<b>0.000</b>	<b>0.000</b>

Values are mean (SD), Mean vitamin D intakes ( $\mu\text{g}/\text{d}$ ) calculated from baseline FFQ. Significant differences between groups of participants are presented in bold. *P* values <0.05 from Mann-Whitney U test.

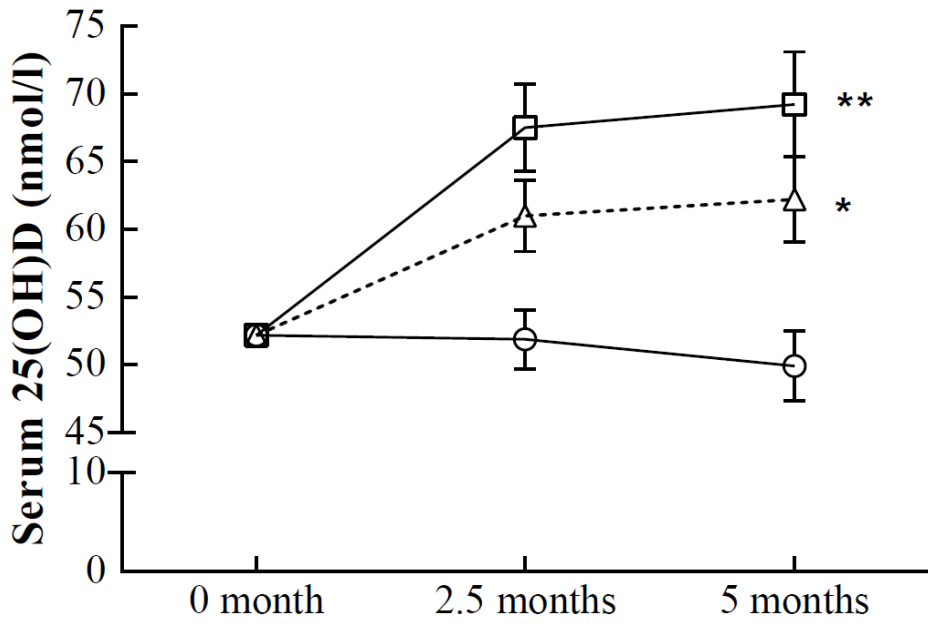
Range from diet: 1.5-29.9  $\mu\text{g}/\text{d}$  (East African women); 1.1-18.6  $\mu\text{g}/\text{d}$  (Finnish women). Range from supplement: 0.0-60.0  $\mu\text{g}/\text{d}$  (East African women); 0.0-57.5  $\mu\text{g}/\text{d}$  (Finnish women).

Range of total intake: 1.5-89.9  $\mu\text{g}/\text{d}$  (East African women); 1.1-68.2  $\mu\text{g}/\text{d}$  (Finnish women)





(a) ○ Placebo    -△- 10 μg    -□- 20 μg



(b) ○ Placebo    -△- 10 μg    -□- 20 μg

