A randomized, double-blinded, placebo-controlled, parallel trial of vitamin D_3 supplementation in adult patients with migraine

Gazerani, Parisa; Fuglsang, Randi; Pedersen, J. G.; Sørensen, J.; Kjeldsen, J. L.; Yassin, H.; Nedergaard, B. S.

Published in:
Current Medical Research and Opinion

DOI (link to publication from Publisher):
10.1080/03007995.2018.1519503

Publication date:
2019

Document Version
Accepted author manuscript, peer reviewed version

Current Medical Research and Opinion

ISSN: 0300-7995 (Print) 1473-4877 (Online) Journal homepage: http://www.tandfonline.com/loi/icmo20

A randomized, double-blinded, placebo-controlled, parallel trial of vitamin D_3 supplementation in adult patients with migraine

P Gazerani, R Fuglsang, JG Pedersen, J Sørensen, JL Kjeldsen, H Yassin & BS Nedergaard

To cite this article: P Gazerani, R Fuglsang, JG Pedersen, J Sørensen, JL Kjeldsen, H Yassin & BS Nedergaard (2018): A randomized, double-blinded, placebo-controlled, parallel trial of vitamin D_3 supplementation in adult patients with migraine, Current Medical Research and Opinion, DOI: 10.1080/03007995.2018.1519503

To link to this article: https://doi.org/10.1080/03007995.2018.1519503

Accepted author version posted online: 05 Sep 2018.

A randomized, double-blinded, placebo-controlled, parallel trial of vitamin D_3 supplementation in adult patients with migraine

Running head: Vitamin D supplementation in migraine

Authors: P Gazerani[*1], R Fuglsang[1], JG Pedersen[1], J Sørensen[1], JL Kjeldsen[1], H Yassin[1], BS Nedergaard[2]
Address: [1]Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Aalborg, Denmark, [2]Center for Clinical and Basic Research (CCBR), Aalborg, Denmark
* Correspondence: Parisa Gazerani, Biomedicine, Department of Health Science and Technology, Faculty of Medicine, Aalborg University, Fredrik Bajers Vej 3B, room 2-205, DK-9220 Aalborg E, Denmark. Email: gazerani@hst.aau.dk

Transparency.

Funding sources that supported the work: This study was financially supported by FSS grants (grant numbers 031301 and 831302) from the Danish Research Council to PG. Eir (Empowering Industry and Research) at SMI® also partially supported the student's work in this project based on a co-financing time-plan. The supporting organizations did not have any role in study design, data collection, data analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication. No funded writing assistance was used in the creation of this manuscript.

Declaration of financial/other relationships: The authors have no financial/other relationships to disclose. CMRO peer reviewers on this manuscript have no relevant financial or other relationships to disclose.

Author Contributions: P Gazerani and BS Nedergaard designed the final study protocol. R Fuglsang, JG Pedersen, J Sørensen, JL Kjeldsen, H Yassin, performed the tests and collected data. P Gazerani, R Fuglsang, and JG Pedersen analyzed the data. All authors discussed the results for the first draft of the manuscript.

Acknowledgments: We warmly thank all staff, study nurses, and laboratory assistants at CCBR Aalborg for their assistance in practical aspects of the study at the clinic, sampling, and laboratory procedures. We especially thank Morten R. Sørensen, Michael W. Boserup, Lone Kristiansen, Susanne Henriksen, and Maria E. Jensen for their excellent support. Furthermore, we would like to thank Anne Bülow-Olsen and Migrænikerforbundet for their assistance in advertising for recruitment. Gratitude also goes to Hans Ch. Hoeck, Flemming W. Bach, Ali Karshenas, Søren Due Andersen, and Meike Heskamp for their professional input and kind assistance. Brian Edwin Cairns, Lars Arendt-Nielsen, Lone Schødt Andersen are also acknowledged for their input at the initial phases of the study protocol development. This study was not possible without excellent engagement and cooperation of our participants; we would like to extend our special appreciation to all of them.

Abstract

Background: Vitamin D levels have been linked to certain pain states, including migraine. We investigated whether vitamin D supplementation would be beneficial for adult patients with migraine (ClinicalTrials.gov Identifier: NCT01695460).

Methods: A randomized, double-blind, placebo-controlled parallel trial was conducted in migraine patients (36 women and 12 men, 18-65 years of age). A 4-week baseline period was conducted before randomization to 24 weeks of treatment. Participants were assigned to receive D3-Vitamin® (n=24, 18 women and 6 men, 100 µg/day D3-Vitamin®) or placebo (n=24, 18 women and 6 men). Migraine attacks and related symptoms were assessed by self-reported diaries. The response rate (i.e., experiencing a 50 % or greater reduction in migraine frequency from baseline to week 24), change in migraine severity, and number of migraine days were recorded. Changes in migraine-related symptoms, HIT-6™ scores, and pain sensitivity tests (pressure pain threshold and temporal summation) were also evaluated. Serum levels of both 25(OH)D and $1,25(OH)_2D$ were assessed from baseline to week 24.

Results: The number of headache days changed from 6.14±3.60 in the treatment group and 5.72±4.52 in the placebo group at baseline to 3.28±3.24 and 4.93±3.24 by the end of the trial, respectively. Migraine patients on D3-Vitamin® demonstrated a significant decrease ($p<0.001$) in migraine frequency from baseline to week 24 compared with placebo. However, migraine severity, pressure pain thresholds or temporal summation did not show a significant change. 25(OH)D levels increased significantly for the D3-Vitamin® group during the first 12 weeks of treatment. There was no significant change in $1,25(OH)_2D$. No side effects were reported or noted.

Conclusions: D3-Vitamin® was superior to placebo in reducing migraine days in migraine patients. Larger studies are required to confirm that vitamin D_3 might be one of the prophylactic options for adult patients with migraine.

Key words: Vitamin D; migraine; 25(OH)D; $1,25(OH)_2D$; pain; headache

Introduction

Migraine is a highly prevalent, complex primary headache, affecting women more than men [1]. This disabling disorder is characterized by spontaneous headache attacks often accompanied by nausea, vomiting, and sensitivity to light [2, 3]. A large number of migraineurs also experience allodynia, a sensation of pain in response to normally non-noxious stimuli [4]. The exact pathogenesis of migraine is unclear, but the general consensus is that migraine is a result of a complex neurovascular imbalance [5, 6, 7]. Migraine has remained undertreated [8]. Usually, the goal of abortive therapy is to block headache, while the goal of prophylactic therapy is to reduce migraine attack frequency, severity, or disease progression [9]. Some common migraine prophylactics include anticonvulsants, beta-blockers, and tricyclic antidepressants [9]. A number of vitamins, minerals, and medicinal herbs have also been considered, including riboflavin, magnesium, and vitamin D [10, 11]. Insufficiency or deficiency of vitamin D has been linked to several painful disorders, including headaches [12]. In general, however, the results are inconclusive, and causal relationships are not established [12, 13, 14, 15, 16]. Vitamin D insufficiency and deficiency are very common in the general population due to several factors, including inadequate sun exposure, inadequate intake, inadequate internal synthesis, or genetic variances in vitamin D metabolism [17, 18]. We hypothesized that vitamin D3 supplementation in a randomized, parallel, double-blinded, placebo-controlled trial could prove effective in adult patients with migraine.

Materials and Methods

Participants and visits

The study was conducted in accordance with guidelines from Good Clinical Practice and the Declaration of Helsinki and approved by the local Ethics Committee, Region North Jutland, Denmark (N-20120052). The trial was also registered with ClinicalTrials.gov (Identifier: NCT01695460).

Subjects were recruited through advertisement on public notice boards, on Netdoktor.dk and forsog.dk, and furthermore through advertisement on social networks (Facebook). Subjects eligible for screening were screened by the study neurologists at Aalborg Hospital North, Denmark. Prior to enrollment, all subjects provided written informed consent. All follow-up visits took place at CCBR A/S Aalborg, Denmark, within the time period of 2012-2016.

The sample size calculation resulted in N≈20, meaning that 20 migraineurs were needed in each treatment arm to ensure adequate statistical power. To adjust for an expected dropout rate of 20 %, we aimed at recruiting 24 migraineurs in each treatment arm, in total, 48 migraineurs; 36 women and 12 men, taking into account the natural distribution of sexes in the migraine population [1].
Migraine diagnosis was based on criteria set by the International Headache Society for headache classification (ICHD-II). Migraineurs within the age range of 18-65 years with migraine onset before the age of 50 years were included. The exclusion criteria included other neurological or neurodegenerative disorders, medical conditions that could potentially interfere with the study, a history of severe head trauma or stroke, musculoskeletal or

psychiatric disorders, ongoing treatment with digoxin or thiazides, existing hypercalcemia, and use of vitamin D supplementation in doses higher than 10 µg/day. Experiencing aura was not used for exclusion.
For each subject, the study lasted 196 days and consisted of 8 visits at 4-week intervals (± 3 days). This flexibility was applied to allow for holidays and to ensure that subjects were headache-free during visits. Initially, the subjects were followed for a baseline period of 4 weeks, after which they were randomized 1:1 to receive either vitamin D_3 supplementation (100 µg daily) or placebo for 24 weeks. Subjects were required to be headache-free at visits 1, 2, 5, and 8 because this could have an influence on sensory assessments. The visits included blood sampling, the Headache Impact Test 6 (HIT-6^{TM}) questionnaire, measuring vital signs (blood pressure, pulse, and oral temperature), and quantitative sensory tests (pressure pain threshold (PPT) and temporal summation). An overview of these procedures for each visit is shown in Table 1. All procedures were performed by the same members of the study group for each subject.

Subjects kept diaries for each migraine attack that was reviewed by the investigators at each visit. In this diary, patients recorded headache occurrence, duration, and severity. Severity was rated on a 1-2-3 scale, referring to mild (=1), moderate (=2), and severe (=3) headache. Furthermore, the presence of aura, nausea, photo- and phonophobia, and allodynia (feeling pain during nonpainful stimuli, such as brushing hair), and the use of abortive therapy were recorded in the diary. Based on the ethical guidelines, participants continued to use their migraine medications but were asked to record and report it. No restrictions were applied.

Medications

Subjects were randomized to receive 24 weeks of treatment with either vitamin D_3 supplementation (D3-Vitamin®, 100 µg/day) or placebo. The dose and treatment duration chosen in this study were based on the following rationales. The dose should have ensured high enough increases in serum 25(OH)D levels to exclude variations due to diet and exposure to sunlight. An effect should have not been evaluated until a steady state of 25(OH)D had been reached and maintained for some time, and therefore the treatment period was important, as the half-life of vitamin D_3 (2-3 weeks) and hence the time to reach steady state is quite long. The treatment period was also important in evaluating migraine. Treatment periods of three to six months are recommended in migraine trial guidelines as it provides more stable estimates of attack frequency [19]. Women and men were randomized separately to ensure an equal distribution of each sex in each treatment arm. Randomization was performed based on randomly generated codes in blocks of four, i.e., per four subjects, two received D3-Vitamin® and two received placebo. Four tablets were administered orally once per day, each containing either 25 µg vitamin D_3 or placebo (consisting of tablet excipients). Both D3-Vitamin® and placebo tablets were obtained from D3-Pharmacy ApS (Aalborg, Denmark). Placebo tablets and the D3-Vitamin® tablets were identical. Treatment was initiated at week 0. Study medication was handed out 6 times; at weeks 0, 4, 8, 12, 16, and 20, i.e., medication for only 4 weeks at a time. Each pill bottle contained 150 tablets, thus ensuring that there were enough tablets even if a visit was rescheduled for a few days late, e.g., in case that the subject suffered a migraine attack. Pill bottles were returned with leftover tablets at every following visit. A tablet count was performed to follow up on compliance with study medication. The subjects were made aware of the importance of taking the study medication correctly. Subjects were instructed that

any missed dose should be taken the same day, immediately when the subject recalled it. They were not allowed to take it the following day. The agreeable limit of compliance was 85 %, meaning that subjects had to have taken at least 85 % of study medication. Leftover study medication was destroyed following completion of the study. A blinded clinical research assistant who did not take part in any other activities provided pills to participants. We ensured that participants and investigators as well as study staff assessing outcomes at visits or entering data after visits remained blinded to treatments.

It is well recognized that vitamin D has a desirable safety profile and very limited potential side effects [20]. However, adverse events were recorded during the study.

Primary and secondary outcomes

This study was conducted to investigate the efficacy of vitamin D_3 (100 µg/day) against migraine in comparison with placebo. The primary efficacy measures were whether and how migraine frequency and severity (pain intensity) could be altered by vitamin D_3 supplementation. Primary efficacy measures were change in migraine attack frequency, number of days with migraine, and severity. Changes from baseline to the last four weeks of the treatment and through the entire double-blind phase were compared between the two treatment groups. Responders were considered those who had a 50 % or greater reduction in migraine attack frequency from baseline to the last four weeks of treatment.

Secondary efficacy measures included changes from baseline to the last four weeks of treatment in migraine-related symptoms, including aura, nausea, photo- and phonophobia, and allodynia, changes in HIT-6™ scores and responses to quantitative sensory tests.

We also investigated whether and how daily vitamin D_3 supplementation could alter serum levels of vitamin D, both 25(OH)D and $1,25(OH)_2D$. Vitamin D has two forms: vitamin D_2 (ergocalciferol) and D_3 (cholecalciferol), which undergo identical metabolism. The major circulating form of vitamin D is 25(OH)D, which is hydroxylated in the kidneys to the biologically active form $1,25(OH)_2D$, which interacts with the vitamin D receptor. Vitamin D status is determined by measuring the precursor 25(OH)D. There are several reasons for this: $1,25(OH)_2D$ circulates in concentrations that are 1000 times less than 25(OH)D, and 25(OH)D is a more stable vitamin D metabolite because it has a half-life of approximately 2-3 weeks (43,50). Furthermore, $1,25(OH)_2D$ is tightly regulated, and only very small concentrations of 25(OH)D are necessary for maintaining sufficient levels of 1,25(OH)D [21]. Interestingly, no studies have investigated a correlation between $1,25(OH)_2D$ and 25(OH)D before and after supplementation with vitamin D_3 to determine whether supplementation can cause an increase in serum $1,25(OH)_2D$ levels as well as in serum 25(OH)D levels. Therefore, we considered measuring both.

Below, more details are given for the assessments.

Headache Diary

A diary was given to each patient with a guide for completing it. Date and time at onset of the attack and its termination time were included.

For the description of the headache aura and the experienced disturbances, the timing was also asked. Location and intensity of headache were also included (on a scale of 1 to 3, in which 1=mild headache, 2=moderate headache, and 3=severe headache). Other symptoms and a short description of those were also included. To record the presence of allodynia, we asked whether the patients experienced increased pain or unpleasant sensations on the skin and when this occurred (i.e., during the headache or between attacks). Examples of allodynia included combing hair, pulling hair back (e.g., ponytail), shaving face, wearing eyeglasses, wearing contact lenses, wearing earrings, wearing a necklace, wearing tight clothing, taking a shower (when shower water hits facial skin), resting head or face on a pillow, exposure to heat (e.g., cooking, washing face with hot water), and exposure to cold (e.g., using an ice pack, washing face with cold water). Type, dose and frequency of medications taken for migraine were also included in the diary. Data obtained from diaries were extracted and recorded in a case report form (CRF) for each patient.

Headache Impact Test

HIT-6^{TM} was created by QualityMetric Incorporated (USA) as a tool to assess and monitor disease burden, including the impact of migraine on functional health and well-being, i.e., quality of life. The HIT-6^{TM} questionnaire was provided to each subject at three visits during the course of the study (See Table 1). The questionnaire provides a score from 36 to 78, indicating the degree to which the subject's quality of life is affected by migraine. A score above 60 indicates severe impact, 56-59 indicates substantial impact, 50-55 indicates some impact, and a score below 50 indicates only little or no impact of migraine on quality of life. HIT-6^{TM} was used to investigate whether the impact on quality of life by migraine changed during the course of the study.

Quantitative Sensory Tests

PPT and temporal summation were assessed to record hypersensitivity and to determine whether these measures changed during the course of the study, i.e., following treatment. Both tests were performed on each side of the head on the temporal muscle, on both arms, at a point on the belly of the brachioradialis muscle, and on the right leg, at a point located on the belly of the tibialis anterior muscle 5 cm distal to the tibial tuberosity. PPT was performed on a point on the temporal muscle as determined as the most sensitive point for migraineurs [22]. This point is located 1.5 cm above the upper medial edge of the ear and 1 cm anterior from there. For temporal summation, a point on the hairless part of the temple was used. During both tests, the subject was lying comfortably on their side, for test points on the head, and on the back, for test points on the limbs.

At the first visit, the location of the points on the limbs was noted to retrieve the same points at the following visits. To ensure that the same point was used every time, the distance from the radial styloid process to the test point was measured and noted for both arms. On the right leg, the distance from the lateral malleolus of the fibula to the test point was measured. These measurements were then used during the following visits to mark the test points.

Pressure Pain Threshold

The pressure pain threshold is used to assess the minimal amount of pressure needed to evoke a pain response in the subject. PPT was performed using a handheld pressure algometer (Somedic AB, Hörby, Sweden) consisting of a 1 cm^2 rubber-tipped probe. The probe was placed perpendicularly on the test points. Pressure was applied at a rate of 30 kPa/s, and subjects were instructed to push a stop button immediately when they defined the pressure as painful. Pressure stimulation was stopped, and the maximum pressure was noted. Three consecutive PPTs were performed on each test point with a minimum of 10 seconds in between. A mean of the three measurements was calculated for each test point. A 25 % deviation from the mean of the three PPTs was used to eliminate outliers, i.e., If a PPT measurement was outside this limit, a new PPT measurement was performed. The mean value was used for subsequent statistical analysis.

Temporal summation

Temporal summation, a clinical correlate of wind-up related to central sensitization, is a frequency-dependent increase in the excitability of spinal and trigeminal neurons [23, 24]. The test was performed with the use of a customized PinPrick stimulator (Aalborg University, Denmark) that exerts a force of 128 mN. For safety reasons, 128 mN was used as the maximum force. For each test site, all stimuli were applied within an area of 1 cm^2. First, a single stimulus was applied by the PinPrick stimulator. Immediately hereafter, the subject was asked to rate the intensity of the stimuli on a 0 to 10 numerical scale, with 0 being “no pain” and 10 being “worst possible pain”. After a period of 10 seconds, a series of 10 identical punctuate stimuli with a frequency of one stimulation per second were performed, and again, the subject was asked to rate the intensity of the stimuli on the numerical scale. From these ratings, the difference between the single stimulus and the series of stimuli was calculated. The test was performed twice for each test point. The mean of the differences was calculated and used for further statistical analysis. Only data from patients with a change in rating from the single stimulus to the 10 consecutive stimuli were included in the data analysis for temporal summation.

Blood sampling and serum analysis

Blood samples were drawn from an antecubital vein using a hypodermic needle (21G, BD Vacutainer® Safety-Lok™, #368654, Becton Dickinson AB, Stockholm, Sweden) and collected in 5 mL serum separation tubes (BD Vacutainer® SST II Plus, #367955, Becton Dickinson AB, Stockholm, Sweden), while the subject sat in a comfortable position. During visits 1, 2, 5, and 8, three blood samples were drawn, one of which served as a backup sample. The blood samples were turned upside down 5- 6 times manually to ensure that the sample was thoroughly mixed with the clot activator in the serum separation tube. The samples were then left for a minimum of 30 minutes to allow clotting before being centrifuged at 3000 rpm for 10 minutes at 20°C. For each serum separation tube, the supernatant serum was transferred to a transfer tube. The transfer tubes were kept at -80°C until further analysis. Serum was analyzed for 25(OH)D and $1,25(OH)_2D$ by chemiluminescence enzyme immunoassay using an IDS-iSYS Fully Automated Immunoassay System.

Data analysis

Data were analyzed using SPSS Version 22. A P-value ≤ 0.05 was considered statistically significant. Normal distributions of data were assessed by means of the Shapiro-Wilks test. If P>0.05, the data were considered to be normally distributed. An independent-samples *t*-test, or the nonparametric equivalent (Mann-Whitney U test), was used to test for baseline equality and uniformity between the two groups, i.e., the D3-Vitamin® group and the placebo group, regarding age and migraine characteristics.

The responder rate was evaluated using a chi-squared test. Two-sample *t*-tests were used for comparisons between the treatment group and the placebo group regarding remaining primary endpoints. For nonparametric data, the Mann-Whitney U test was applied. Primary efficacy measures were also analyzed in the individual treatment groups by means of the paired samples *t*-test. For nonparametric data, the Wilcoxon signed-rank test was applied. Descriptive data were presented for migraine-related symptoms. McNemar's test was used to assess the presence of migraine-related symptoms before and after treatment in the individual treatment groups. Repeated measures analysis of variance (RM ANOVA) was used for comparison of changes across time within and between the two treatment groups for HIT-6™, quantitative sensory tests, and changes in serum levels of 25(OH)D and $1,25(OH)_2D$. For nonparametric data, Friedman's test was applied. Spearman's correlations for nonparametric data were used to assess potential correlations between serum levels of 25(OH)D and $1,25(OH)_2D$ at baseline and week 24.

Results

This study screened 65 participants, among whom 48 were identified as eligible and enrolled (36 women, 12 men, 18-65 years). Forty-eight subjects completed the study. Participants were randomly assigned to receive D3-Vitamin® (n=24, 18 women and 6 men) or placebo (n=24, 18 women and 6 men). We had 2 dropouts who were both in the placebo group, and discontinuation was due to personal choice and not for any medical reasons; therefore, we replaced these two participants (Flowchart 1). No adverse events were reported, and compliance levels were above 85 %. All subjects were Caucasian, from Nordic countries, and residents of Denmark.

Within- and between-group differences in the use of migraine medication were not significantly different. The type and dose of medications also remained generally stable for both groups. Assessment of blinding at weeks 12 and 24 by James' blinding index [25] showed successful overall blinding.

The demographic and baseline characteristics of the study participants are summarized in Table 2. There were no significant differences between the two groups at baseline, and the baseline mean level of serum 25(OH)D was within the optimal level (75-125 nmol/L).

Primary efficacy measures

Responders were those with a 50 % or greater reduction in migraine attack frequency from baseline to week 24. A significant difference was found between the two groups (P=0.008), with higher responder rates in the D3-vitamin® group. A summary of the primary efficacy measures is presented in Table 3.

From baseline to week 24, the median change in migraine severity on a 1-2-3 scale did not show any statistically significant difference between the two groups (P=0. 823, Mann-Whitney U test). The mean reduction in the number of migraine days from baseline to week 24 was higher in the D3-Vitamin® group compared with the placebo group (P=0.047).

For the D3-Vitamin® group, the Friedman test showed a significant change across time in attack frequency (P=0.014). Regarding the severity of attacks, the Friedman test did not reveal a significant change across time (P=0.621); however, the number of migraine days was affected (P=0.012). For the placebo group, the Friedman test did not show a significant change across time in either attack frequency (P=0.059) or severity (P=0.614). The repeated measures ANOVA for the number of migraine days also did not show a significant change across time in this group (P=0.548).

Secondary efficacy measures

The proportions of subjects experiencing migraine-related symptoms during baseline and week 24 in the individual treatment groups were evaluated, and no significant change was observed (McNemar's test, Table 4).

The mean HIT-6™ scores for each treatment group at each time point were evaluated. Repeated measures ANOVA with treatment as the between subject factor was conducted to compare HIT-6™ scores between the D3-Vitamin® group and the placebo group over time. There was no significant main effect across time (P=0.520). No significant difference was evident in the change across time between the two treatment groups (P=0.227). However, in both groups, the score showed a decrease over time. For the D3-Vitamin® group, it was from

63.25±4.17 from first measure (visit 2) to 53 in last (visit 8), and for the placebo group, it was from 62.75±5.31 to 56.83.

Quantitative sensory tests were employed to assess hyperalgesia and central sensitization. Repeated measures ANOVA and the nonparametric equivalent Friedman test showed that there were no significant changes in PPT levels at any test or time point, neither for the D3-Vitamin® group nor the placebo group (P>0.05). For temporal summation, repeated measures ANOVA and the Friedman test showed that there was no significant change across time at any test point (P>0.05).

Serum analysis

For 25(OH)D, a repeated measures ANOVA with Greenhouse-Geisser correction revealed a significant main effect for time (P<0.001) and a significant difference in the change across time between the two treatment groups (P<0.001). In the D3-Vitamin® group, there was an increase in mean serum 25(OH)D levels at week 24 compared with baseline. This increase across time was statistically significant (P=0.003, the Friedman test). The major change in the D3-Vitamin® group occurred between the start of intervention and week 12 (Wilcoxon's signed-rank test, P=0.043), i.e., during the first 12 weeks of treatment. After week 12, the rate of increase in serum 25(OH)D levels leveled off, and there was no significant change from week 12 to week 24 (Wilcoxon's signed-rank test, P=0.080). The mean serum 25(OH)D levels in the placebo group did not show a significant change from baseline to week 24 (P=0.781, repeated measures ANOVA).

We also tested whether a correlation existed between serum levels of 25(OH)D and $1,25(OH)_2D$ at baseline and at week 24. According to Spearman's rho, there was no significant correlation between 25(OH)D and $1,25(OH)_2D$ at baseline (P=0.384) or at week 24 (P=0.285). We performed both unadjusted analyses and adjusted analysis for baseline values, age, and body mass index. Regression models indicated similar outcomes independent of baseline values, age, or body mass index.

Discussion

This study demonstrated that D3-Vitamin® was superior to placebo in reducing migraine attacks in adults with migraine.

Headache and associated characteristics

A Norwegian study assessed vitamin D status in patients with musculoskeletal pain, fatigue and headache [15]. Vitamin D deficiency was found in 58 % of those patients. A cross-sectional study also showed that in a British population, chronic widespread pain was associated with low levels of 25(OH)D in females. However, no such association was evident for male subjects [26]. In contrast, the European Male Ageing Study (n=3075) found that subjects classified as having either chronic widespread pain or "other pain" had significantly lower mean levels of 25(OH)D [27]. We did not find any vitamin D deficiency among our participants at the start of the study. However, all participants were diagnosed with migraine based on the IHC criteria. Therefore, we tested our hypothesis that vitamin D supplementation might have an effect on migraine and demonstrated that D3-Vitamin® was superior to placebo in the reduction of migraine attacks in adults with migraine.

Previously, two case report studies had shown that vitamin D was effective in reducing the frequency of migraine in four women [28, 29]. One of these studies included two premenopausal women suffering from menstrual migraine and premenstrual syndrome, and the other included two postmenopausal women suffering from migraine. All four subjects were treated with a combination of vitamin D2 or D3 and calcium in varying doses. During these studies, all four subjects reported a decrease in frequency and duration of migraine attacks. The two premenopausal women also reported a relief of premenstrual syndrome, including related pain symptoms. Our findings also demonstrated a decrease in the frequency of migraine. A recent study has

also demonstrated that simvastatin and vitamin D were effective in preventing headache in adults with episodic migraine [30].

Although the nature of our study does not allow for a discussion of the exact mechanism underlying the observed effect, one explanation might be the anti-inflammatory effects of vitamin D, which could affect neuroinflammation associated with migraine [31]. Studies have shown that vitamin D, at physiologic levels, can suppress the production of proinflammatory cytokines such as IL-6 and tumor necrosis factor-α in human monocytes and macrophages [32]. In relation to this, it has been shown that in migraine patients, serum levels of these proinflammatory cytokines are higher compared to healthy controls [33, 34]. Vitamin D has also been shown to cause an increase in the production of the anti-inflammatory cytokine IL-10 by cells that are involved in the immune response [35]. IL-10 has been shown to be lower in migraine patients compared to healthy controls [34]. Vitamin D, in the active form $1,25(OH)_2D$, can inhibit the synthesis of inducible nitric oxide synthase, which generates NO [36, 37]. In relation to this, it has been shown that NO can stimulate the synthesis and release of CGRP from trigeminal ganglion neurons, and it is known that CGRP stimulates the release of NO from trigeminal ganglion cells. Thus, this might lead to a positive feedback loop that can enhance and maintain inflammatory processes within the sensory ganglion, which could contribute to sensitization of meningeal nociceptors during migraine [38, 39]. Taken together, these findings suggest that vitamin D has an anti-inflammatory effect that could influence migraine.

Analysis of migraine-related symptoms, i.e., aura, nausea, photo- and phonophobia, and allodynia showed no significant pattern of change across time for either group (D3-Vitamin® and placebo) in this study. The quality of life questionnaire HIT-6™ showed that both groups were severely affected by migraine at baseline, with the D3-Vitamin® group having slightly, but not significantly, higher baseline scores compared with the placebo group. Both groups improved during the treatment period, but the reduction was not significant. The fact that both groups presented a similar pattern might be explained by the placebo effect that is often observed in migraine trials [40]. Quantifying the placebo response in migraine indicates that these responses are common in migraine, especially in parallel-designed studies of prophylactic agents [40]. When applying HIT-6™ scores as a measure of quality of life in studies regarding migraine prophylactics, it should be considered that HIT-6™ scores are affected greatly by the severity of migraine attacks and not so much by the frequency [41]. Therefore, another quality of life assessment tool in combination with HIT-6™ might be better, for example, the migraine disability assessment (MIDAS) questionnaire.

Pressure pain threshold and temporal summation

The PPT test was employed to investigate whether PPT changed during the treatment period, i.e., whether PPT was affected by vitamin D_3 supplementation. In this study, PPT was assessed as a measure of hyperalgesia, which has been demonstrated in migraineurs [22]. However, in this study, there was no significant change across time, for either the D3-Vitamin® group or the placebo group. Temporal summation was also assessed as the clinical correlate of wind-up, which is related to central sensitization. Similar to the PPT results, there was no significant change across time for either the D3-Vitamin® group or the placebo group at any test point. A study has demonstrated the presence of higher temporal summation in migraineurs between attacks

compared with healthy controls [42]. The authors suggested that migraineurs have altered membrane excitability and hence increased pain production, which is also present between attacks.

Serum analysis for 25(OH)D and 1,25(OH)2D levels

Serum 25(OH)D levels increased significantly following vitamin D_3 supplementation. The most notable increase occurred between week 0 and week 12, and the rate of increase then leveled off from week 12 to 24. The results showed a significant increase in serum 25(OH)D levels in the treatment group. However, no significant change in serum $1,25(OH)_2D$ was observed. As $1,25(OH)_2D$ has a half-life of approximately four to six hours [43], this might explain why no change was observed across the four time points that only represent snapshots in sampling time. When looking at raw data, there is great variation between individual subjects and within subjects at the different time points. $1,25(OH)_2D$ was measured to investigate whether there was a correlation between serum levels of the two metabolites. To the best of our knowledge, this has not previously been investigated before and after vitamin D_3 supplementation. Only small concentrations of 25(OH)D are necessary for maintaining sufficient levels of $1,25(OH)_2D$ [21]. However, if severe vitamin D deficiency (25(OH)D) was observed, then $1,25(OH)_2D$ might also be deficient. We also did not find a correlation between $1,25(OH)_2D$ and 25(OH)D (neither at baseline nor following supplementation with vitamin D_3). If a positive correlation had been observed following vitamin D_3 supplementation, concern might have been raised in relation to the risk of developing hypercalcemia [20]. There is a risk of side effects manifested as hypercalcemia when high doses of vitamin D_3 are administered. However, the risk is considered low with regard to the dose administered in this study (100 µg/day), since several studies involving healthy men and women receiving this dose did not report hypercalcemia, even when supplementation was administered for longer periods of up to 14 months [44, 45]. Because the risk was considered low in this study, calcium was not measured.

In our study, the D3-Vitamin® group reached serum levels above the optimal level; however, serum levels were still well below those considered potentially toxic. None of our participants reported any adverse effects, and the treatments were well tolerated. Vitamin D_3 is considered safe and has a very limited side effect profile [20] in comparison with existing migraine prophylactic agents. Vitamin D_3 supplementation is also relatively inexpensive and is available as an over-the-counter medication in many regions. Hence, it is an attractive agent for the prophylaxis of migraine.

Study limitations

Participants in this study were all Danish, balanced at enrollment and had similar migraine features. Therefore, although the main finding is promising, studies in a larger and more diverse population are required to identify whether this finding can be replicated. In this population, we did not have enough power to analyze sex-related differences in the efficacy of D3-Vitamin® versus placebo. It should be noted that migraine attacks might be triggered by various factors. Hormonal changes, stress, not eating, and sleep disturbances are among them [46, 47]. Trigger factors were not used for any subanalyses in this study.

In prophylactic migraine trials, most studies permit migraine medication, as recommended by guidelines [19]. In this study, migraine medication was also allowed. Two of our subjects were on concomitant prophylactic

therapy, and they did not alter the dosage or regimen during the study (one subject received Lamictal® and propranolol, and one subject received amitriptyline).

Conclusion

A significant reduction in the frequency of attacks over time was evident in migraine patients treated with D3-Vitamin®. Migraine-related symptoms were not significantly affected by the supplementation, nor were pain thresholds and sensitivity affected. Quality of life, measured by HIT-6™, did not show a significant improvement. One hundred micrograms/day D3-Vitamin® was effective in raising serum 25(OH)D levels. No change was observed in serum $1,25(OH)_2D$ levels. Considering the reduction of headache frequency and safety profile of D3-Vitamin® observed in this study, we propose considering this agent as one of the prophylactic options in adult patients with migraine. This needs to be proven in a larger trial.

References

1. Stovner LJ, Andree C. Prevalence of headache in Europe: a review for the Eurolight project. J Headache Pain. 2010 Aug;11(4):289-99. doi: 10.1007/s10194-010-0217-0. PubMed PMID: 20473702; PubMed Central PMCID: PMCPMC2917556.
2. Charles A. The evolution of a migraine attack - a review of recent evidence. Headache. 2013 Feb;53(2):413-9. doi: 10.1111/head.12026. PubMed PMID: 23278169.
3. Ward TN. Migraine diagnosis and pathophysiology. Continuum (Minneap Minn). 2012 Aug;18(4):753-63. doi: 10.1212/01.CON.0000418640.07405.31. PubMed PMID: 22868539.
4. Burstein R, Yarnitsky D, Goor-Aryeh I, et al. An association between migraine and cutaneous allodynia. Ann Neurol. 2000 May;47(5):614-624. doi: Doi 10.1002/1531-8249(200005)47:5<614::Aid-Ana9>3.0.Co;2-N. PubMed PMID: WOS:000086731000009; English.
5. Chen ZY, Chen XY, Liu MQ, et al. Altered functional connectivity of amygdala underlying the neuromechanism of migraine pathogenesis. Journal of Headache and Pain. 2017 Jan 23;18:1-8. doi: 10.1186/s10194-017-0722-5. PubMed PMID: WOS:000393667300001; English.
6. D'Andrea G. Pathogenesis of chronic migraine: the role of neuromodulators. Journal of Headache and Pain. 2015 Dec;16. doi: Artn A38

10.1186/1129-2377-16-S1-A38. PubMed PMID: WOS:000368633600039; English.
7. Vecchia D, Pietrobon D. Migraine: a disorder of brain excitatory-inhibitory balance? Trends Neurosci. 2012 Aug;35(8):507-520. doi: 10.1016/j.tins.2012.04.007. PubMed PMID: WOS:000307622600006; English.
8. Diener HC, Solbach K, Holle D, et al. Integrated care for chronic migraine patients: epidemiology, burden, diagnosis and treatment options. Clin Med (Lond). 2015 Aug;15(4):344-50. doi: 10.7861/clinmedicine.15-4-344. PubMed PMID: 26407383.
9. Rizzoli PB. Acute and preventive treatment of migraine. Continuum (Minneap Minn). 2012 Aug;18(4):764-82. doi: 10.1212/01.CON.0000418641.45522.3b. PubMed PMID: 22868540.

10. Adams J, Barbery G, Lui CW. Complementary and alternative medicine use for headache and migraine: a critical review of the literature. Headache. 2013 Mar;53(3):459-73. doi: 10.1111/j.1526-4610.2012.02271.x. PubMed PMID: 23078346.
11. Karakurum Goksel B. The Use of Complementary and Alternative Medicine in Patients with Migraine. Noro Psikiyatr Ars. 2013 Aug;50(Suppl 1):S41-S46. doi: 10.4274/npa.y6809. PubMed PMID: 28360583; PubMed Central PMCID: PMCPMC5353077.
12. Prakash S, Kumar M, Belani P, et al. Interrelationships between chronic tension-type headache, musculoskeletal pain, and vitamin D deficiency: Is osteomalacia responsible for both headache and musculoskeletal pain? Ann Indian Acad Neurol. 2013 Oct;16(4):650-8. doi: 10.4103/0972-2327.120487. PubMed PMID: 24339599; PubMed Central PMCID: PMCPMC3841620.
13. Knutsen KV, Madar AA, Brekke M, et al. Effect of vitamin D on musculoskeletal pain and headache: A randomized, double-blind, placebo-controlled trial among adult ethnic minorities in Norway. Pain. 2014 Dec;155(12):2591-8. doi: 10.1016/j.pain.2014.09.024. PubMed PMID: 25261164.
14. Yang Y, Zhang HL, Wu J. Is headache related with vitamin D insufficiency? J Headache Pain. 2010 Aug;11(4):369; author reply 371. doi: 10.1007/s10194-010-0235-y. PubMed PMID: 20602247; PubMed Central PMCID: PMCPMC3476355.
15. Knutsen KV, Brekke M, Gjelstad S, et al. Vitamin D status in patients with musculoskeletal pain, fatigue and headache: a cross-sectional descriptive study in a multi-ethnic general practice in Norway. Scand J Prim Health Care. 2010 Sep;28(3):166-71. doi: 10.3109/02813432.2010.505407. PubMed PMID: 20642395; PubMed Central PMCID: PMCPMC3442332.
16. Al-Nimer MS. Vitamin D: Is it a primary hormone targeting the migraine headache or just as adjunct therapy? Neurosciences (Riyadh). 2017 Jan;22(1):69. doi: 10.17712/nsj.2017.1.20160561. PubMed PMID: 28064336.
17. Holick MF. The vitamin D deficiency pandemic and consequences for nonskeletal health: mechanisms of action. Mol Aspects Med. 2008 Dec;29(6):361-8. doi: 10.1016/j.mam.2008.08.008. PubMed PMID: 18801384; PubMed Central PMCID: PMCPMC2629072.
18. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010 Jul 17;376(9736):180-188. doi: 10.1016/S0140-6736(10)60588-0. PubMed PMID: WOS:000280313100025; English.
19. Tfelt-Hansen P, Pascual J, Ramadan N, et al. Guidelines for controlled trials of drugs in migraine: third edition. A guide for investigators. Cephalalgia. 2012 Jan;32(1):6-38. doi: 10.1177/0333102411417901. PubMed PMID: 22384463.
20. Hathcock JN, Shao A, Vieth R, et al. Risk assessment for vitamin D. Am J Clin Nutr. 2007 Jan;85(1):6-18. PubMed PMID: 17209171.
21. Cianferotti L, Marcocci C. Subclinical vitamin D deficiency. Best Pract Res Clin Endocrinol Metab. 2012 Aug;26(4):523-37. doi: 10.1016/j.beem.2011.12.007. PubMed PMID: 22863394.
22. Fernandez-de-las-Penas C, Madeleine P, Cuadrado ML, et al. Pressure pain sensitivity mapping of the temporalis muscle revealed bilateral pressure hyperalgesia in patients with strictly unilateral migraine. Cephalalgia. 2009 Jun;29(6):670-6. doi: 10.1111/j.1468-2982.2008.01831.x. PubMed PMID: 19891059.

23. Eide PK. Wind-up and the NMDA receptor complex from a clinical perspective. Eur J Pain. 2000;4(1):5-15. doi: 10.1053/eujp.1999.0154. PubMed PMID: 10833550.
24. Filatova E, Latysheva N, Kurenkov A. Evidence of persistent central sensitization in chronic headaches: a multi-method study. J Headache Pain. 2008 Oct;9(5):295-300. doi: 10.1007/s10194-008-0061-7. PubMed PMID: 18690491; PubMed Central PMCID: PMCPMC3452198.
25. Bang H, Ni L, Davis CE. Assessment of blinding in clinical trials. Control Clin Trials. 2004 Apr;25(2):143-56. doi: 10.1016/j.cct.2003.10.016. PubMed PMID: 15020033.
26. Atherton K, Berry DJ, Parsons T, et al. Vitamin D and chronic widespread pain in a white middle-aged British population: evidence from a cross-sectional population survey. Ann Rheum Dis. 2009 Jun;68(6):817-22. doi: 10.1136/ard.2008.090456. PubMed PMID: 18697776.
27. McBeth J, Pye SR, O'Neill TW, et al. Musculoskeletal pain is associated with very low levels of vitamin D in men: results from the European Male Ageing Study. Ann Rheum Dis. 2010 Aug;69(8):1448-52. doi: 10.1136/ard.2009.116053. PubMed PMID: 20498201.
28. Thysjacobs S. Alleviation of Migraines with Therapeutic Vitamin-D and Calcium. Headache. 1994 Nov-Dec;34(10):590-592. doi: DOI 10.1111/j.1526-4610.1994.hed3410590.x. PubMed PMID: WOS:A1994PX07800008; English.
29. Thys-Jacobs S. Vitamin D and calcium in menstrual migraine. Headache. 1994 Oct;34(9):544-6. PubMed PMID: 8002332.
30. Buettner C, Nir RR, Bertisch SM, et al. Simvastatin and Vitamin D for Migraine Prevention: A Randomized, Controlled Trial. Ann Neurol. 2015 Dec;78(6):970-981. doi: 10.1002/ana.24534. PubMed PMID: WOS:000367678100013; English.
31. Garcion E, Wion-Barbot N, Montero-Menei CN, et al. New clues about vitamin D functions in the nervous system. Trends Endocrin Met. 2002 Apr;13(3):100-105. doi: Pii S1043-2760(01)00547-1

Doi 10.1016/S1043-2760(01)00547-1. PubMed PMID: WOS:000174350500005; English.
32. Zhang Y, Leung DYM, Richers BN, et al. Vitamin D Inhibits Monocyte/Macrophage Proinflammatory Cytokine Production by Targeting MAPK Phosphatase-1. Journal of Immunology. 2012 Mar 1;188(5):2127-2135. doi: 10.4049/jimmunol.1102412. PubMed PMID: WOS:000300610800012; English.
33. Perini F, D'Andrea G, Galloni E, et al. Plasma cytokine levels in migraineurs and controls. Headache. 2005 Jul-Aug;45(7):926-931. doi: DOI 10.1111/j.1526-4610.2005.05135.x. PubMed PMID: WOS:000230670500013; English.
34. Uzar E, Evliyaoglu O, Yucel Y, et al. Serum cytokine and pro-brain natriuretic peptide (BNP) levels in patients with migraine. Eur Rev Med Pharmaco. 2011 Oct;15(10):1111-1116. PubMed PMID: WOS:000296917400001; English.
35. Chambers ES, Hawrylowicz CM. The impact of vitamin D on regulatory T cells. Curr Allergy Asthma Rep. 2011 Feb;11(1):29-36. doi: 10.1007/s11882-010-0161-8. PubMed PMID: 21104171.
36. Garcion E, Nataf S, Berod A, et al. 1,25-Dihydroxyvitamin D3 inhibits the expression of inducible nitric oxide synthase in rat central nervous system during experimental allergic encephalomyelitis. Brain Res Mol Brain Res. 1997 May;45(2):255-67. PubMed PMID: 9149100.

37. Garcion E, Sindji L, Montero-Menei C, et al. Expression of inducible nitric oxide synthase during rat brain inflammation: regulation by 1,25-dihydroxyvitamin D3. Glia. 1998 Mar;22(3):282-94. PubMed PMID: 9482214.
38. Messlinger K, Lennerz JK, Eberhardt M, et al. CGRP and NO in the trigeminal system: mechanisms and role in headache generation. Headache. 2012 Oct;52(9):1411-27. doi: 10.1111/j.1526-4610.2012.02212.x. PubMed PMID: 22788114.
39. Strecker T, Dux M, Messlinger K. Nitric oxide releases calcitonin-gene-related peptide from rat dura mater encephali promoting increases in meningeal blood flow. J Vasc Res. 2002 Nov-Dec;39(6):489-96. doi: 67206. PubMed PMID: 12566974.
40. Macedo A, Banos JE, Farre M. Placebo response in the prophylaxis of migraine: a meta-analysis. Eur J Pain. 2008 Jan;12(1):68-75. doi: 10.1016/j.ejpain.2007.03.002. PubMed PMID: 17451980.
41. Sauro KM, Rose MS, Becker WJ, et al. HIT-6 and MIDAS as measures of headache disability in a headache referral population. Headache. 2010 Mar;50(3):383-95. doi: 10.1111/j.1526-4610.2009.01544.x. PubMed PMID: 19817883.
42. Weissman-Fogel I, Sprecher E, Granovsky Y, et al. Repeated noxious stimulation of the skin enhances cutaneous pain perception of migraine patients in-between attacks: clinical evidence for continuous sub-threshold increase in membrane excitability of central trigeminovascular neurons. Pain. 2003 Aug;104(3):693-700. doi: 10.1016/S0304-3959(03)00159-3. PubMed PMID: WOS:000185101600029; English.
43. Holick MF. Vitamin D Status: Measurement, Interpretation, and Clinical Application. Ann Epidemiol. 2009 Feb;19(2):73-78. doi: 10.1016/j.annepidem.2007.12.001. PubMed PMID: WOS:000263388800001; English.
44. Barger-Lux MJ, Heaney RP, Dowell S, et al. Vitamin D and its major metabolites: serum levels after graded oral dosing in healthy men. Osteoporos Int. 1998;8(3):222-30. doi: 10.1007/s001980050058. PubMed PMID: 9797906.
45. Tjellesen L, Hummer L, Christiansen C, et al. Serum concentration of vitamin D metabolites during treatment with vitamin D2 and D3 in normal premenopausal women. Bone Miner. 1986 Oct;1(5):407-13. PubMed PMID: 2849489.
46. Andress-Rothrock D, King W, Rothrock J. An analysis of migraine triggers in a clinic-based population. Headache. 2010 Sep;50(8):1366-70. doi: 10.1111/j.1526-4610.2010.01753.x. PubMed PMID: 21044280.
47. Kelman L. The triggers or precipitants of the acute migraine attack. Cephalalgia. 2007 May;27(5):394-402. doi: 10.1111/j.1468-2982.2007.01303.x. PubMed PMID: 17403039.

Tables

Table 1. An overview of procedures performed at each visit

	Visit 1 Week -4	Visit 2 Week 0	Visit 3 Week 4	Visit 4 Week 8	Visit 5 Week 12	Visit 6 Week 16	Visit 7 Week 20	Visit 8 Week 24
Duration	60	60	20	20	60	20	20	60
Blood	x	x			x			x
HIT-6™		x			x			x
Vital signs	x	x	x	x	x	x	x	x
QST	x	x			x			x

HIT-6™: Headache Impact Test 6, Vital signs: Blood pressure, pulse, and temperature; QST (quantitative sensory tests): Pressure pain threshold and temporal summation. Duration of visits is indicated in minutes.

Flowchart 1. Patient flow diagram.

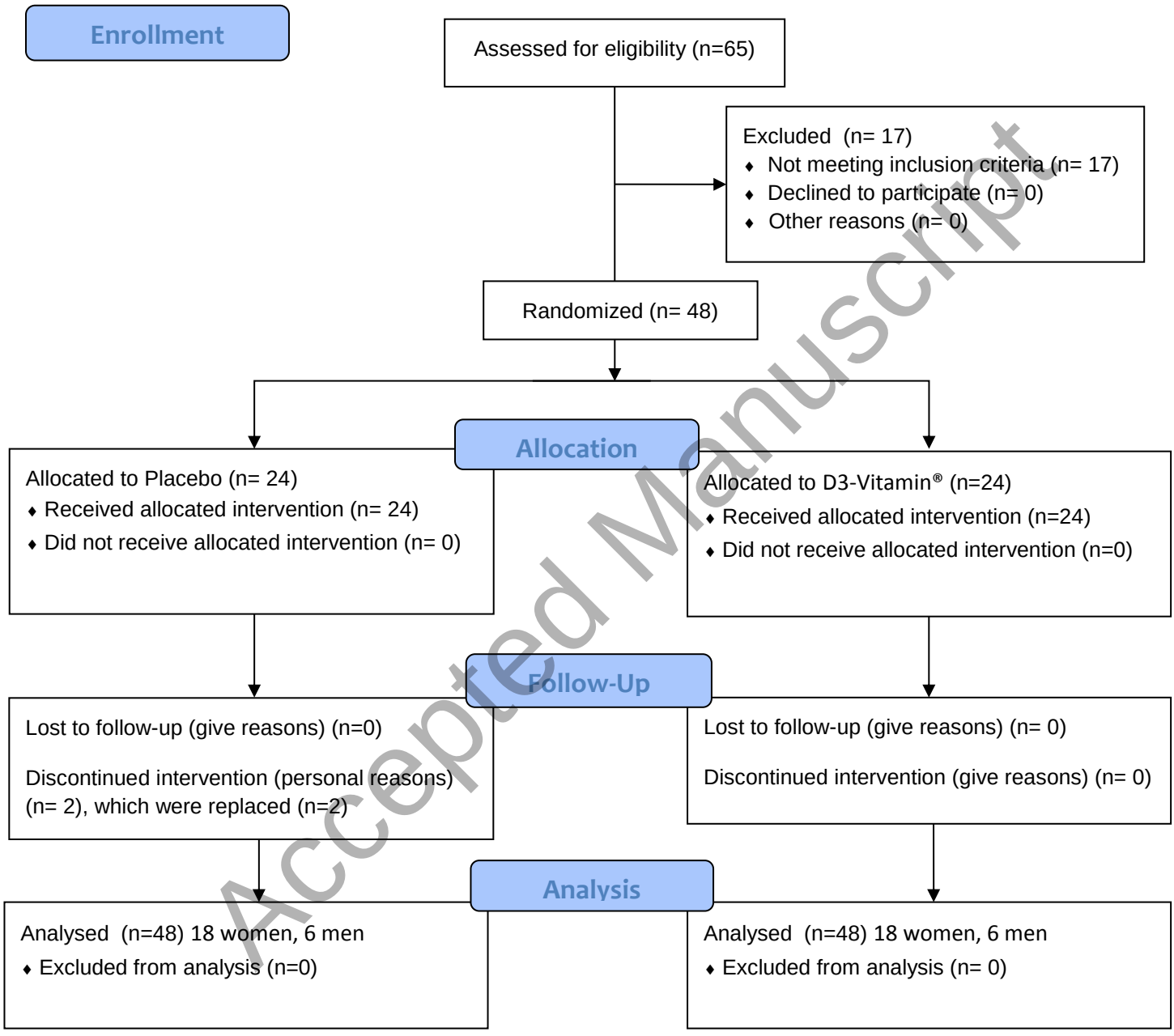

Table 2. Demographic characteristics of the study participants at baseline

Characteristics at baseline	D3-Vitamin® group (n=24) 18 women, 6 men	Placebo group (n=24) 18 women, 6 men	P value
Age (years)	45.5±12.1	43.7±10.2	0.634
Frequency of migraine attacks	4.0	4.0	0.802
Severity of migraine attacks	2.64±1.20	2.38±1.90	0.831
Number of migraine days	6.14±3.60	5.72±4.52	0.322
HIT-6™ score	63.25±4.17	62.75±5.31	0.872
25(OH)D (nmol/L)	87.43±32.00	78.96±30.86	0.548
$1,25(OH)_2D$ (pg/mL)	43.55±10.57	43.71±11.60	0.834

Except data for frequency (median), all data are shown as mean ± SD (standard deviation), HIT-6™ = Headache Impact Test-6. P-values are given for comparison of baseline levels between the two groups, and analyzed using an independent samples *t*-test. For frequency of migraine attacks, non-parametric equivalent, Mann-Whitney U test, was utilized. $P \leq 0.05$ are considered statistically significant.

Table 3: Summary of primary efficacy measures

Primary outcome	D3-Vitamin® group (n=24) 18 women, 6 men	Placebo group (n=24) 18 women, 6 men
Attack frequency		
Week 0	3.00±1.07	3.75±1.98
Week 1-4	2.73±1.23	2.33±2.13
Week 5-8	2.88±1.25	3.12±1.88
Week 9-12	1.53±1.66	3.68±1.06
Week 13-16	1.38±1.77	3.70±1.22
Week 17-20	1.26±1.72	3.93±2.51
Week 21-24	1.29±1.03	3.57±1.92
Attack severity		
Week 0	2.16±0.45	2.29±0.53
Week 1-4	2.26±0.27	2.16±0.23
Week 5-8	2.12±0.14	2.33±1.23
Week 9-12	1.88±1.07	2.19±0.58
Week 13-16	1.64±1.24	2.15±0.54
Week 17-20	1.73±1.05	1.83±1.82
Week 21-24	1.87±1.32	1.79±0.95
Number of days with migraine		
Week 0	6.25±4.00	6.13±3.36
Week 1-4	4.88±1.96	5.25±3.77
Week 5-8	4.15±2.32	5.88±2.85
Week 9-12	3.75±3.37	4.75±1.83

Week 13-16	3.88±2.21	4.75±1.25
Week 17-20	3.89±3.64	4.53±3.78
Week 21-24	3.28±3.24	4.93±3.24

Attack severity was rated as 1=mild headache, 2=moderate headache, and 3=severe headache. Data are presented as mean±SD (standard deviation).

Table 4: Presence of migraine-related symptoms

Migraine related symptoms	D3-Vitamin® group (n=24) 18 women, 6 men	Placebo group (n=24) 18 women, 6 men
Aura		
Baseline	12. 2%	12.5%
Week 21-24	10.4%	10.3%
P-value	0.534	0.367
Nausea		
Baseline	27.3%	26.4%
Week 21-24	25.9%	26.1%
P-value	0.574	0.531
Photophobia		
Baseline	9.5%	8.4%
Week 21-24	8.1%	7.2%
P-value	0.721	0.834
Phonophobia		
Baseline	4.4%	3.8%
Week 21-24	3.7%	4.1%
P-value	0.545	0.457
Allodynia		
Baseline	21.7%	20.5%
Week 21-24	19.6%	18.8%
P-value	0.574	0.631

P-values are given for a change in the presence of migraine related symptoms from baseline to week 21-24 for the individual treatment groups (McNemar's test).