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Effect of vitamin D and omega-3 on nocturnal enuresis of 7-15-year-old children

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Summary

Introduction: Nocturnal enuresis is known to be a common urinary bladder complication in children. Recent studies have associated vitamin D and omega-3 insufficiency with nocturnal enuresis.

Aim: This was a 2-month randomized, placebo-controlled, double-blind trial to measure the effects of vitamin D, omega-3 supplements, and their combination on nocturnal enuresis among 7-15-year-old children.

Materials and Methods: Participants (180 children with nocturnal enuresis) were selected from children referred to the Pediatric ward of Imam Reza Clinic in Shiraz, Iran. Demographic information and anthropometric measures were taken. Fasting blood and urine samples were also collected, and serum vitamin D and urine prostaglandin E2 were measured before and after intervention. Enuretic children were randomly assigned to four groups: Group A, vitamin D (1000 IU/day); Group B, omega-3 (1000 mg/day); Group C, both omega-3 (1000 mg/day) and vitamin D (1000 IU/day); and Group D, placebo.

Results: The results suggested that the study groups were not significantly different regarding demographic and anthropometric measures at baseline. Comparison of different groups revealed that 44.4% of participants in Group A, 28.2% of Group B and 45% of Group C were cured from enuresis ($P=0.03$). Serum 25(OH) D was significantly increased in Group A, but urine prostaglandin E2 was not significantly decreased in response to supplementation. Although
supplementation with both Group A and Group B were found to be effective, the combined intervention did not increase the effect of each intervention solely.

**Conclusions:** The results showed that supplementation with vitamin D and omega-3 could reduce the number of wet nights among 7-15-year-old children with nocturnal enuresis.

**Keywords:** Enuresis; Vitamin D; Omega-3 fatty acids; Prostaglandin E2

**Summary table.** The effect of treatments on nocturnal enuresis in the four study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Not cured</th>
<th>Cured</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Group A (vitamin D)</td>
<td>20 (55.6%)</td>
<td>16 (44.4%)</td>
<td>36 (100%)</td>
</tr>
<tr>
<td>Group B (omega-3)</td>
<td>28 (71.8%)</td>
<td>11 (28.2%)</td>
<td>39 (100%)</td>
</tr>
<tr>
<td>Group C (vitamin D + omega-3)</td>
<td>22 (55%)</td>
<td>18 (45%)</td>
<td>40 (100%)</td>
</tr>
<tr>
<td>Group D (placebo)</td>
<td>31 (91.2%)</td>
<td>3 (8.8%)</td>
<td>34 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>101 (67.8%)</td>
<td>48 (32.2%)</td>
<td>149 (100%)</td>
</tr>
</tbody>
</table>

\[P = 0.03\]
Introduction

Nocturnal enuresis is a common disorder that may lead to several social and personal problems for both children and their parents [1]. The prevalence of primary nocturnal enuresis (children who have never been consistently dry through the night) is 15% in 5-year-old children and is twice as common in boys than girls. The number of wet nights reduces as the child gets older [2]. Although the mechanism of nocturia is not adequately known, it is believed that there are several factors involved such small bladder, high nocturnal urine production, and the child’s inability to awaken at night [3]. Data on Iranian children indicate that 8% of primary school and preschool children are suffering from nocturnal enuresis [4].

Medical treatment for enuresis among children is potentially effective providing the affected children follow a treatment program. Simple behavioral intervention and arousal alarm are other options that are used as first-line non-pharmacologic treatment for nocturnal enuresis. However, the main necessity in behavioral intervention is parental involvement and child motivation, which are not easily achieved [5]. Pharmacotherapy (i.e. the use of desmopressin, imipramine and nortriptyline) is the second step. Although some studies have shown that drugs could reduce wet nights/weeks, which is the desired outcome, the effect is unsustainable after withdrawal [6] and some side effects have been reported, such as hyponatremia [7], postural hypotension, dry mouth, constipation, perspiration, tachycardia, nausea, lethargy, and insomnia [8]. These findings show that new therapeutic options are needed to safely and effectively keep enuretic children dry for longer periods of time.
Recent studies suggest that non-pharmacological treatments such as nutritional therapy may be effective in children with enuresis. Evidence has revealed that vitamin D and omega-3 insufficiency are risk factors for enuresis [9,10], so their supplementation may be a potential solution for this disorder. Recent studies have shown that vitamin D deficiency can be the reason for nocturnal enuresis in children. Vitamin D has an important role in calcium homeostasis in the kidney: in the proximal tubule, 1,25-dihydroxyvitamin D (1,25(OH)\textsubscript{2}D) can modify the calcium-sensing receptor gene and regulate calcium excretion, which indirectly affects fluid retention [11]. In addition, researchers have documented that there is a link between nocturia and obstructive sleep apnea (OSA) [12], which is a common disorder among children and is the outcome of low serum 25(OH) D levels [13]. So, vitamin D supplementation may provide a solution to treat hypercalciuria, which has been reported to be associated with nocturnal enuresis [14], and it may also reduce sleep disorders, which are an occasional cause of enuresis.

Polyunsaturated fatty acids such as omega-3 constitute an environmental factor that is able to act on central nervous system function. Thus, omega-3 deficiency can affect the brain and nervous system development, which can cause prematurity of the nervous system and abnormal urination [15]. Also, omega-3 fatty acids may influence primary nocturnal enuresis by regulation of prostaglandin E2, nitric oxide synthesis, and brain signaling [9]. There is evidence that omega-3 is a potent inhibitor of prostaglandin E2 synthesis [16]. Studies have shown that the effect of prostaglandins on the urinary bladder is dose-dependent relaxation and contraction. An in vitro study documented that prostaglandin E series, especially prostaglandin E2, causes vesical smooth muscle contraction and urethral relaxation [17,18]. Thus, omega-3 may prevent instability and over-contractility of the bladder muscles through reduction of prostaglandin E2 synthesis. On the other hand, some studies have suggested that the effect of omega-3 on
nocturnal enuresis may involve an interaction with nitric oxide synthesis [19]. Omega-3 may reduce wet nights through possible effects on the bladder and urethral contraction by inhibiting nitric oxide synthesis. This evidence indicates that omega-3 supplementation may be an alternative treatment for nocturnal enuresis.

Therefore, according to the suggested mechanisms of vitamin D and omega-3 in the urinary tract, the present study was designed to test the hypothesis that vitamin D and omega-3 would decrease pediatric nocturnal enuresis compared to placebo.

Materials and methods

A double-blinded, randomized, placebo-controlled trial was conducted from summer 2015 through winter 2016 in the Pediatric ward of Imam Reza Clinic, Iran. Children were selected if they were 7-15 years old, were suffering from nocturnal enuresis for at least 3 months, had three or more wet nights per week, had normal serum calcium levels (8.8-10.8 mg/dl), and a normal calcium to creatinine ratio (<0.2). Participants were excluded if they did not start the treatment or their parents wanted to withdraw them from the study. Participants were randomly allocated to four study arms via randomized block design, each consisting of 30 children. Neither the physicians nor the participants were aware of the allocation and the interventions attached to them. The tablets and capsules were stored in alphabetically labeled (coded) bottles. The coding procedure was conducted by one of the clinic staff out of the research team. A code was then assigned to each participant. The participants were unblended at the end of the eighth week of the study period.
The routine treatment regimen and parent-child education for enuresis was given to all participants before their allocation as the first-line treatment [20]. All participants received a tablet and a capsule. The first group (Group A, \(n=36\)) received a vitamin D tablet (1000 IU/day) and an omega-3 gel-free capsule (placebo) per day. The second group (Group B, \(n=38\)) received an omega-3 gel capsule (1000 mg, amount/1000 mg: ecosapantanoic acid (EPA) 180 mg, docosahexaenoic acid (DHA) 120 mg) and a vitamin D-free tablet (placebo) per day. The third group (Group C, \(n=35\)) received an omega-3 gel capsule (1000 mg) and a tablet of vitamin D (1000 IU) per day. The fourth group (Group D Control, \(n=34\)) received a vitamin D-free tablet (placebo) and an omega-3 gel-free capsule (placebo) per day (Fig. 1). All participants in each group received the interventions for 8 weeks.

Doses were determined based on literature suggesting that 1000 IU/day vitamin D and 1000 mg/day omega-3 for 2 months is safe and effective [21-23]. Vitamin D tablets (1000 IU) were obtained from Jalinus company, Iran, and vitamin D placebo supplements were provided by Shiraz School of Pharmacy, Iran. Both omega-3 and placebo capsules were supplied by Zahravi pharmacy factory, Iran.

Information on age, sex, bowel habits, number of wet nights/week, urination/day, and family history of enuresis was obtained from an interview with each child’s mother. Anthropometric measures (e.g. weight, height, waist-to-hip ratio, mid-upper-arm circumference (MUAC)) were obtained during the first visit to the clinic, according to the World Health Organization’s protocol [24]. Details of the measurements can be found in Appendix 1.

Mothers reported wet nights after they occurred. The participants and their parents were given an oral and written explanation of the study protocol, including benefits and procedures of
intervention at the beginning of the study. The parents read and signed an informed consent
document.

Blood and urine samples were collected and analyzed at the beginning and the end of the
8-week intervention period. Blood and urine samples were taken in morning after at least 8 hours
of fasting. Blood samples were centrifuged and serum was separated immediately. Serum and
urine samples were kept at –80 °C for further analysis. Serum and urine calcium and urine
creatinine were measured by a BT1500 autoanalyzer using calcium and creatinine (Diasys
diagnostic system, Germany) kit via spectrophotometry method. The 25(OH) D was measured
using 25(OH) D (Monobind, USA) kit via Enzyme-linked Immunosorbent Assay (ELISA)
method. Prostaglandin E2 levels were measured using urine prostaglandin E2 kit (Crystalday,
China) using ELISA method.

Priori calculation suggested that 30 patients were needed in each group to detect more
than 15% success in the treatment (less than three wet nights per week) of nocturnal enuresis in
at least one of the intervention groups compared to placebo. The data were analyzed by SPSS,
version 16. Chi-squared and ANOVA tests were used to compare the groups for background
variables. To compare the cure rate among the study groups, Chi-squared (univariate analysis)
was applied. However, despite randomization, due to a significant between-group differences in
the number of nocturnal enuresis before intervention, logistic regression (multivariable analysis)
was also applied to control for the effect of this important factor. The level of significance (P-
value) was set at 0.05.
Results

The total number of study participants was 180. In total, 18 children had to be excluded at the beginning because the children refused to co-operate after consent was obtained from their parents and before the defined interventions were to be started. As the result, the study was started with 162 enuretic children (98 boys and 64 girls) with the mean age of 8.5 years. Fifty percent of the study participants had received no conventional treatment for enuresis, and of those who had received any treatment, 5.6% responded to the treatment.

As Table 1 shows, at baseline, age, sex, bowel habits, number of wet nights/week, urination/day, and family history of enuresis were not significantly different among the study groups. Also, weight, height, waist-to-hip ratio, and MUAC of the participants were similar among the groups. Moreover, the analysis of biochemical characteristics showed that children in study groups had similar serum vitamin D and urine prostaglandin E2 levels at baseline. The results suggested that 43.6% of the participants had a positive family history of nocturnal enuresis. Moreover, 22.2% of the children had urinary incontinence, but there was no significant between-group difference in diurnal enuresis and the number of wet nights per week ($P>0.05$). In addition, 12.3% of the enuretic children were suffering from constipation.

As presented in Table 2, more than half of the children in Groups A, B and C were cured after the intervention period ($P=0.03$). Treatment with vitamin D was also associated with significantly higher serum 25(OH)D (35.5 ng/ml versus 27 ng/ml) levels ($P=0.03$). As presented in Table 3, urine prostaglandin E2 levels did not significantly decreased in the omega-3 supplementation group (Group B). Compared to the other groups, fewer wet nights/week were
observed in Group B compared with the other groups. Finally, of the 149 participants, enuresis completely disappeared in 10.7%, and 32 participants (21.5%) had less than two wet nights/week at the end of the study.

**Discussion**

Nocturnal enuresis is a disorder among children that has a high incidence rate and causes behavioral disorders and also sleep problems [25]. Hashem et al. found prevalence of nocturnal enuresis to be 18.7% among school-aged children, which was higher than other studies in Iran [26]. Inability to control urination at night causes behavioral disturbances and is a highly stressful condition for enuretic children as compared with their healthy peers [27]. Nocturnal enuresis is caused by many different factors. It spontaneously disappears in 15% of children each year, but it should be cured if it disturbs the child [5].

Analysis of the current study showed that the average serum 25(OH) vitamin D was 26.14 ng/ml among the study population, which was significantly higher than the normal value (20 ng/ml) before the intervention. Luanluan Li et al. showed that more than half of enuretic children (70.1%) participating in their study had sufficient serum 25(OH) vitamin D, and they found a negative relationship between serum 25(OH) vitamin D and the incidence of nocturnal enuresis; they also found that higher serum 25(OH) vitamin D resulted in lower incidence of enuresis [10]. The current study showed that vitamin D supplementation significantly reduced the number of wet nights in children with nocturnal enuresis, while it increased serum 25(OH)
vitamin D levels after 2 months of intervention. The effect of vitamin D treatment on nocturnal enuresis might have been greater if the follow-up had been longer.

After 2 months of intervention, the analysis demonstrated that omega-3 supplements at a dosage of 1000 mg/day did not have an impact on prostaglandin E2 secretion, but it did reduce the mean number of wet nights/week. Previously, Kuznetsova et al. showed that prostaglandins are involved in the pathogenesis of enuresis. Their results showed that sodium excretion and nocturnal urine production increased in children with enuresis, which was not due to higher nocturnal urinary excretion of prostaglandins E1 and E2 and F alpha [28]. However, the study conducted by Loang et al. showed that omega-3 was a potential treatment for primary nocturnal enuresis. On the other hand, DHA, the most abundant omega-3 fatty acid in the brain, plays an important role in neuronal processes including neurogenesis, neuroplasticity, neuron differentiation and survival, membrane integrity, synaptogenesis and expression of synapsins and glutamate receptors [29]. Therefore, nocturnal enuresis, which is an abnormal reflex of micturition, may occur as a result of functional immaturity of the central nervous system and a lack of proper inhibition, which occurs in an area linked to the micturition center [9]. Also, within the urinary system most of the effects of nitric oxide are similar to those of prostaglandin E2: nitric oxide inhibits sodium and fluid reabsorption and decreases anti-diuretic hormone production [30]. Therefore, the effect of omega-3 on prostaglandin production and other probable mechanisms such as nervous system development and production of a proper startle response and inhibition of nitric oxide production in the kidney (with the same effect of prostaglandin E2 on the kidney tubules) make omega-3 supplementation a probable treatment for nocturnal enuresis [9]. The current study suggests that the decrease in wet nights/week in omega-3 supplemented children is not related to changes in prostaglandin E2 secretion and excretion.
However, consumption of both vitamin D and omega-3 supplements had more effect on enuretic children, but because treatment occurred in vitamin D consumers (with or without omega-3 supplementation) it is suggested that vitamin D has the therapeutic effect. No adverse effect was reported in the study population.

Conclusion

Based on these study results, vitamin D and omega-3 may have therapeutic values for nocturnal enuresis in children. The mechanism of their operation for reduction of wet nights in enuretic children was still unclear and evidence of improvement with vitamin D was stronger. Further studies are required to determine the dosage of vitamin D and omega-3 supplements that could be more effective; it is suggested that future studies should follow children to assess the rate of relapse after withdrawal. No clinical trial could be found that used vitamin D supplements to correct urinary incontinence, so it is recommended, for the first time, that vitamin D supplementation may treat enuretic children.

The present study had a number of limitations. There are several mechanisms that explain the positive influence of omega-3 supplementation on nocturnal enuresis, and in this trial only one mechanism could be followed (prostaglandin E2 excretion). Also, it would have been better if the participants could have been followed for more than 2 months of study, and if the children’s conditions could have been recorded after withdrawal.
Conflict of interest: None.

Acknowledgements: The authors would like to thank Vice chancellor of Research Shiraz University of Medical Sciences, Shiraz, Iran for financial support and also Center for Development of Clinical Research of Nemazee Hospital and Dr. Nasrin Shokrpour for editorial assistance.

Ethical Approval: The study protocol and ethical aspects were approved by the Human Ethics Committee of the Research Council of the Dean of Research Affairs of the Shiraz University of Medical Sciences. The study was registered in Iranian clinical trial web site (www.irct.ir IRCT2015062222868N1).

Appendix 1

Height was measured to the nearest 0.5 cm using a non-stretchable tape fixed on the wall while the child was standing without shoes, and buttocks and shoulders touching the wall. Weight was measured by a digital scale (Glamor BS-801, Hitachi, China) without shoes and light dressing. Waist and hip circumferences were measured to the nearest half centimeter. Waist circumference was measured at mid-distance between the last floating rib and the iliac crest at the end of normal expiration with a standard tape measure to the nearest 0.5 cm. Hip circumference was measured at the maximal protrusion of the buttocks, by a nonelastic flexible tape to the nearest 0.5 cm. Waist circumference was divided by the hip to determine the waist-to-hip ratio (WHR).
The mid upper arm circumference (MUAC) was measured using a non-stretchable tape at the midpoint of the non-dominant arm between the olecranon and acromion with the child’s arm relaxed to the nearest 0.1 cm.

References


20. Katz ER, DeMaso DRN. Rumination, Pica, and Elimination (Enuresis, Encopresis)


Table 1. Demographic and anthropometric characteristics of the study participants.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vitamin D (Group A) n=36 (%)</th>
<th>Omega-3 (Group B) n=39 (%)</th>
<th>Vitamin D + omega-3 (Group C) n=40 (%)</th>
<th>Placebo (Group D) n=34 (%)</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>22 (57.9%)</td>
<td>27 (69.2%)</td>
<td>29 (59.2%)</td>
<td>20 (55.6%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>16 (42.1%)</td>
<td>12 (30.8%)</td>
<td>20 (40.8%)</td>
<td>16 (44.4%)</td>
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<td>Constipation</td>
<td>No</td>
<td>34 (89.5%)</td>
<td>34 (87.2%)</td>
<td>43 (87.8%)</td>
<td>31(86.1%)</td>
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<td>Yes</td>
<td>4 (10.5%)</td>
<td>5 (12.8%)</td>
<td>6 (12.2%)</td>
<td>5 (13.9%)</td>
</tr>
<tr>
<td>Family history of enuresis</td>
<td>No</td>
<td>15 (39.5%)</td>
<td>22 (56.4%)</td>
<td>26 (53.1%)</td>
<td>12 (33.3%)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>23 (60.5%)</td>
<td>17 (43.6%)</td>
<td>23 (46.9%)</td>
<td>24 (66.7%)</td>
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<tr>
<td>Mean±SD</td>
<td>Age (years)</td>
<td>8.65±2.10</td>
<td>8.25±2.22</td>
<td>8.29±2.10</td>
<td>8.72±2.67</td>
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<tr>
<td></td>
<td>Weight (kg)</td>
<td>27.44±7.91</td>
<td>26.17±9.55</td>
<td>28.25±11.50</td>
<td>31.26±13.54</td>
</tr>
<tr>
<td></td>
<td>Height (cm)</td>
<td>129.98±13.91</td>
<td>124.06±13.50</td>
<td>126.34±15.07</td>
<td>128.27±17.34</td>
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<td></td>
<td>Waist to hip ratio (cm)</td>
<td>0.84±0.06</td>
<td>0.83±0.06</td>
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<td>MUAC (cm)</td>
<td>20.45±2.41</td>
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<td>19.62±2.83</td>
<td>19.84±2.96</td>
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<td>Urination/day</td>
<td>2.53±5.39</td>
<td>2.52±5.48</td>
<td>2.47±5.65</td>
<td>2.83±6.56</td>
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<td></td>
<td>Baseline nocturnal enuresis/week*</td>
<td>*5 (2-7)</td>
<td>*7 (3-7)</td>
<td>*7 (1-7)</td>
<td>*7 (3-7)</td>
</tr>
</tbody>
</table>

*Mode (range)

MUAC, mid-upper-arm circumference
Table 2. The effect of treatments on nocturnal enuresis in the four study groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Not cured</th>
<th>Cured</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$ (%)</td>
<td>$n$ (%)</td>
<td></td>
</tr>
<tr>
<td>Group A (vitamin D)</td>
<td>20 (55.6%)</td>
<td>16 (44.4%)</td>
<td>36 (100%)</td>
</tr>
<tr>
<td>Group B (omega-3)</td>
<td>28 (71.8%)</td>
<td>11 (28.2%)</td>
<td>39 (100%)</td>
</tr>
<tr>
<td>Group C (vitamin D + omega-3)</td>
<td>22 (55%)</td>
<td>18 (45%)</td>
<td>40 (100%)</td>
</tr>
<tr>
<td>Group D (placebo)</td>
<td>31 (91.2%)</td>
<td>3 (8.8%)</td>
<td>34 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>101 (67.8%)</td>
<td>48 (32.2%)</td>
<td>149 (100%)</td>
</tr>
</tbody>
</table>

$P=0.03$
**Table 3.** Change (post-pre intervention) in children’s serum vitamin D and urine prostaglandin E2 levels.

<table>
<thead>
<tr>
<th></th>
<th>Mean of post-pre±SD</th>
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<tr>
<td><strong>Serum 25(OH)D (ng/ml)</strong></td>
<td></td>
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<tr>
<td>Group A (vitamin D)</td>
<td>7.5±8.15</td>
</tr>
<tr>
<td>Group B (omega-3)</td>
<td>2.99±4.83</td>
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<td>Group C (vitamin D + omega-3)</td>
<td>8.06±8.66</td>
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<td>Group D (placebo)</td>
<td>3.7±5.97</td>
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<td><em>P</em>-value</td>
<td>0.005</td>
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<tr>
<td><strong>Urine prostaglandin E2 (pg/ml)</strong></td>
<td></td>
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<tr>
<td>Group A (vitamin D)</td>
<td>5.14±44.40</td>
</tr>
<tr>
<td>Group B (omega-3)</td>
<td>8.11±29.9</td>
</tr>
<tr>
<td>Group C (vitamin D + omega-3)</td>
<td>10.02±47.43</td>
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<tr>
<td>Group D (placebo)</td>
<td>6.45±27.04</td>
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<tr>
<td><em>P</em>-value</td>
<td>0.36</td>
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Assessed for eligibility
(n=180)

Excluded
(n=18)
Refused to participate
(n=18)

Randomized
(n=162)

Vitamin D
(n=40)

Analysed
(n=36)
Lost
(n=4)

Omega-3
(n=40)

Vitamin D + omega-3
(n=40)

Placebo
(n=40)

Analysed
(n=34)
Lost
(n=6)

Analysed
(n=39)
Lost
(n=1)

Analysed
(n=40)
Lost
(n=0)

Fig. 1. Participant flow.