

## ORIGINAL ARTICLE

# The impact of 1-year vitamin D supplementation on vitamin D status in athletes: a dose–response study

EMP Backx<sup>1</sup>, M Tieland<sup>1</sup>, K Maase<sup>2</sup>, AK Kies<sup>3</sup>, M Mensink<sup>1</sup>, LJC van Loon<sup>4</sup> and LCPGM de Groot<sup>1</sup>

**BACKGROUND/OBJECTIVES:** To assess the prevalence of vitamin D deficiency in Dutch athletes and to define the required dosage of vitamin D<sub>3</sub> supplementation to prevent vitamin D deficiency over the course of a year.

**SUBJECTS/METHODS:** Blood samples were collected from 128 highly trained athletes to assess total 25(OH)D concentration. Of these 128 athletes, 54 male and 48 female athletes (18–32 years) were included in a randomized, double blind, dose–response study. Athletes with either a deficient (< 50 nmol/l) or an insufficient (50–75 nmol/l) 25(OH)D concentration were randomly assigned to take 400, 1100 or 2200 IU vitamin D<sub>3</sub> per day orally for 1 year. Athletes who had a total 25(OH)D concentration above 75 nmol/l at baseline continued with the study protocol without receiving vitamin D supplements. Serum total 25(OH)D concentration was assessed every 3 months, as well as dietary vitamin D intake and sunlight exposure.

**RESULTS:** Nearly 70% of all athletes showed an insufficient (50–75 nmol/l) or a deficient (< 50 nmol/l) 25(OH)D concentration at baseline. After 12 months, serum 25(OH)D concentration had increased more in the 2200 IU/day group (+50 ± 27 nmol/l) than the sufficient group receiving no supplements (+4 ± 17 nmol/l; *P* < 0.01) and the 1100 IU/day group (+25 ± 23 nmol/l; *P* < 0.05).

Supplementation with 2200 IU/day vitamin D resulted in a sufficient 25(OH)D concentration in 80% of the athletes after 12 months.

**CONCLUSIONS:** Vitamin D deficiency is highly prevalent in athletes. Athletes with a deficient or an insufficient 25(OH)D concentration can achieve a sufficient 25(OH)D concentration within 3 months by taking 2200 IU/day.

*European Journal of Clinical Nutrition* (2016) 70, 1009–1014; doi:10.1038/ejcn.2016.133; published online 27 July 2016

## INTRODUCTION

Vitamin D inadequacy is traditionally defined as a serum total 25-hydroxyvitamin D (25(OH)D) concentrations below 50 nmol/l based on studies concerning bone health and calcium metabolism.<sup>1</sup> Using this definition, only 40–60% of the athletes have adequate total 25(OH)D concentrations.<sup>2–4</sup> More recent studies show that low total 25(OH)D concentrations are also associated with reduced muscle strength<sup>5</sup> and muscle protein synthesis,<sup>6</sup> which may impact athletic performance. So far, a limited number of studies have investigated the effect of vitamin D supplementation on athletic performance.<sup>7–9</sup> These studies demonstrate that increasing serum total 25(OH)D concentration improves sprint capacity and vertical jump performance,<sup>7</sup> enhances muscular strength recovery following intense exercise<sup>8</sup> as well as decreases the risk of musculoskeletal injuries.<sup>9</sup> However, these studies tend to have a small sample size and did not control for covariates such as physical activity, which might explain the positive effects that are found.

Data from both observational and intervention studies demonstrate improvements in athletic performance when serum total 25(OH)D concentrations are above 75 nmol/l.<sup>7–11</sup> However, it is important to note that not all studies have confirmed this finding,<sup>3,12</sup> and more well-controlled studies are needed to assess the optimal 25(OH)D concentrations specifically for athletic performance. An effective and commonly used strategy to increase serum total 25(OH)D concentrations is supplementation with vitamin D<sub>3</sub>.<sup>13</sup> Although a number of dose–response studies have been performed, most have investigated older, non-athletic

populations.<sup>14–16</sup> The results from these studies cannot be directly translated to athletes considering differences in dietary vitamin D intake,<sup>17–19</sup> body composition,<sup>20,21</sup> sunlight exposure<sup>17,19</sup> and the age-related capacity of the skin to produce vitamin D.<sup>22</sup> Moreover, to improve a low total 25(OH)D concentration at the end of the winter and to maintain a sufficient 25(OH)D concentration throughout the year, studies need to focus on assessing total 25(OH)D concentration during all seasons. Therefore, the current study was conducted to (1) assess the prevalence of vitamin D deficiency (< 50 nmol/l) in a group of highly trained athletes and (2) identify the dose of vitamin D<sub>3</sub> supplementation required to achieve and maintain a total 25(OH)D concentration > 75 nmol/l throughout the course of a year.

## MATERIALS AND METHODS

### Subjects

A total of 128 highly trained athletes were recruited through contacts with their coaches, trainers and/or medical doctors. All athletes participated on a national or an international level in sports supported by Netherlands Olympic Committee\**Netherlands Sports Confederation (NOC\*NSF)* and were all living in the Netherlands at a latitude of ~52°N. Athletes were excluded if they had chronic diseases that could influence total 25(OH)D concentrations and/or the response to vitamin D supplementation. Athletes were also excluded if they used vitamin D supplements and participants were not allowed to start taking supplements during the study. Athletes who used a sun bed or travelled to foreign countries were not excluded, but this information was monitored by questions in the

<sup>1</sup>Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands; <sup>2</sup>Netherlands Olympic Committee\**Netherlands Sports Confederation (NOC\*NSF)*, Arnhem, The Netherlands; <sup>3</sup>DSM Biotechnology Center, Delft, The Netherlands and <sup>4</sup>NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, The Netherlands. Correspondence: Professor LCPGM de Groot, Division of Human Nutrition, Wageningen University, Bomenweg 4, P.O. Box 8129, NL-6700EV Wageningen, The Netherlands.

E-mail: Lisette.deGroot@wur.nl

Received 6 October 2015; revised 23 May 2016; accepted 24 June 2016; published online 27 July 2016

sunlight questionnaire. At baseline, athletes were asked to fill in a training log with the amount of training hours per day for 1 week.

**Design and intervention**

The present study consisted of two parts. The first part was a cross-sectional observation of total 25(OH)D concentration at the end of the winter to assess the prevalence of vitamin D deficiency in athletes. The second part comprised a dose-response study to compare the effect of three dosages of vitamin D supplements on total 25(OH)D concentrations in a selection of athletes who participated in the first part.

After providing written informed consent, 58 male and 70 female athletes had their serum total 25(OH)D concentration assessed, while concurrently recording vitamin D and calcium intake, sun exposure and general well-being (baseline measurement). Baseline measurements were assessed at the end of the winter season (March until April 2013), as the winter months are known to impose the highest risk of vitamin D deficiency due to the lack of sun exposure.<sup>23</sup> From this group, 102 athletes were included in a randomized, double-blind, dose-response study for 12 months. Serum 25(OH)D, vitamin D and calcium intake and sun exposure were reassessed every 3 months (Figure 1). Selected athletes were stratified based on their baseline total 25(OH)D concentration as having a deficient (< 50 nmol/l), insufficient (50–75 nmol/l) or sufficient (> 75 nmol/l) total 25(OH)D concentration. Those athletes with a deficient (n=43) and an insufficient (n=46) total 25(OH)D concentration were randomized to take 400 (n=31), 1100 (n=29) or 2200 IU (n=29) vitamin D<sub>3</sub> (cholecalciferol) per day for 1 year (Table 1). The sufficient group (n=13) continued with the study protocol without receiving vitamin D supplements.

For subjects whose total 25(OH)D concentration reached 125 nmol/l or higher (as measured during the 3-month assessment), the dose was adjusted to a maintenance dose of 400 IU/day to prevent possible harmful effects.<sup>1</sup>

The study was approved by the Medical Ethics Committee of Wageningen University (12/09). The design and aim of the study were registered in the NIH clinical trial database (ClinicalTrials.gov number: NCT02008201).

**Supplements**

Vitamin D supplements (400, 1100 or 2200 IU) were taken on a daily basis together with a meal to enhance absorption.<sup>24</sup> Vitamin D<sub>3</sub> was provided by DSM Biotechnology Center (Delft, The Netherlands) in a capsulated form. Capsules were administered in a double-blind manner and provided in blisters. Supplements were tested by independent laboratories for the correct dosage and to guarantee the absence of contamination with prohibited substances. The HFL Sport Science lab guaranteed the absence of contamination with prohibited substances. The dosages were measured by TNO Triskelion BV by the following method: Samples are saponified using a 1.5 mol/l potassium hydroxide ethanolic solution. The mixture is extracted using diisopropyl ether. The extract is partly evaporated and redissolved in methanol. After sterol precipitation and solid phase extraction, the eluate is further purified by fractionation with straight

phase chromatography. Finally, the resulting extract is analysed by reversed phase chromatography with diode array detection, quantifying at 265 nm.

Subjects were asked to fill in a calendar to monitor compliance to the supplement intake. Also, subjects were asked to hand in all supplement packages. For statistical analysis, all subjects were included. The calendar and supplement count data were used to explain deviating results of individual subjects. Overall, 4 subjects in the 2200 IU/day group displayed deviating 25(OH)D concentrations. For these subjects, the calendar and supplement count helped explain the data.

**Measurements**

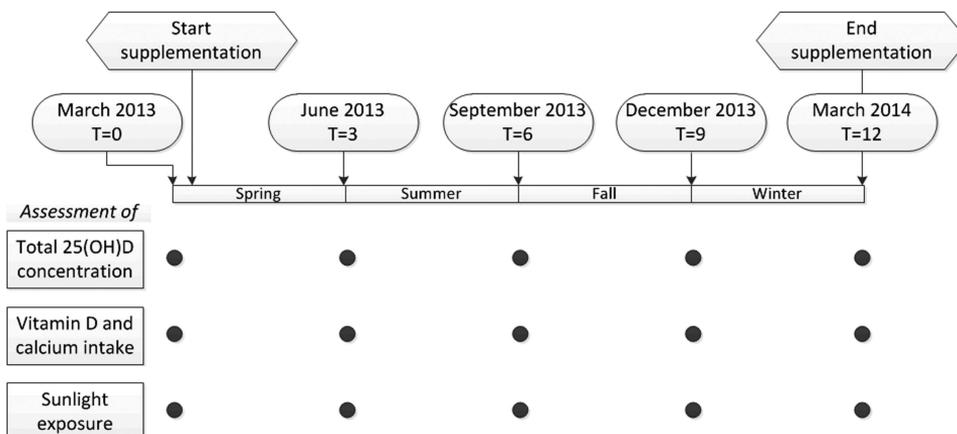
*Serum 25(OH)D.* Subjects were fasted for at least 3 h before blood samples were collected by venipuncture to assess serum total 25(OH)D concentration. After collection, blood tubes (SST Tube with Silica Clot Activator, Becton, Dickinson and Company, Franklin Lakes, NJ, USA) were stored for at least 30 min in a dark box to allow clotting, after which they were centrifuged for 10 min at 1550 G. Serum was separated and stored at -80 °C until analysis. Serum 25(OH)D concentrations were analysed with a liquid chromatography–tandem mass spectrometry (LC–MS/MS) assay (Online SPE) at the VU Medical Centre, Amsterdam. The VU Medical Centre participates in the United Kingdom National External Quality Assessment Schemes (UK NEQAS) and the Dutch society for quality control in medical laboratories (SKML). The intra-assay and inter-assay coefficients of variability of this lab were 5–9 and 7–9%, respectively.

**Safety**

To prevent possible harmful effects of the vitamin D supplements, athletes in the 2200 and 1100 IU/day group who reached total 25(OH)D concentrations higher than 125 nmol/l switched to a maintenance dose of 400 IU/day. This occurred in 31 athletes. In only 5 of them, total 25(OH)D concentrations dropped below 75 nmol/l (lowest value 47 nmol/l) at the end of the winter (T=12).

**Table 1.** Block randomisation of vitamin D supplementation

Vitamin D status	Total 25(OH)D concentration (nmol/l)	Supplementation (IU/day)
Deficient	< 50	400
		1100
		2200
Insufficient	50–75	400
		1100
		2200
Sufficient	> 75	No supplements



**Figure 1.** Overview of the study design.

Vitamin D and sun exposure questionnaire

Vitamin D and calcium intake were calculated from a validated 56-item food frequency questionnaire.<sup>25</sup> Sun exposure was measured by a questionnaire that assessed the time spent outdoors likely representing exposure to sunlight. The questions pertained to time spent outdoors during the last 2 weeks to 3 months. The self-reported sun exposure score could range from 0 to 108 and was used to rank athletes. Scores obtained at T0, T9 and T12 were recoded to 0 to accommodate for the absence of cutaneous synthesis of vitamin D during the winter months. Body weight and height were also assessed by a questionnaire, and BMI was calculated as body weight in kg/(height in m).<sup>2</sup>

Statistics

A sample size calculation was performed prior to the start of the study using GPower.<sup>26</sup> To detect a significant difference in serum 25(OH)D in response to the lowest and middle doses of vitamin D supplementation with an 1-beta of >0.80 and an alpha of <0.05, at least 10 participants per group were required.<sup>27</sup> Considering a drop-out rate of 30%, 15 participants per group were needed. To include a group of 90 subjects with a deficient or an insufficient total 25(OH)D concentration, we estimated that 120–140 athletes should be included at baseline.<sup>28</sup>

Data are presented as means ± s.d.. Intention-to-treat analysis was applied, meaning that all subjects were included also in case they missed one or more measurements. Baseline characteristics and total 25(OH)D concentrations were normally distributed as evaluated by plotting a histogram and the skewness and kurtosis values (all between -1.0 and +1.0). To check whether there were baseline differences between the groups, a one-way ANOVA was used in combination with a Bonferroni *post hoc* test to assess the differences between specific dosage groups. Linear mixed models analysis in combination with a Bonferroni *post hoc* test was applied to assess the differences between groups over time. Supplement dose, time and supplement dose × time were entered as fixed factors. Random factors were included based on a significant contribution to the outcome in the linear mixed model. The following random factors were tested: vitamin D intake, gender, age, skin colour, indoor/outdoor athletes, baseline 25(OH)D, exposure to sunlight and BMI. Only baseline 25(OH)D and exposure to sunlight were significant. First, linear mixed models were applied with only exposure to sunlight as random factors to compare

mean total 25(OH)D concentrations between groups (Table 3A). Second, exposure to sunlight and baseline total 25(OH)D concentrations were included as random factors to compare the changes over time (Table 3B). Data were analysed using SPSS version 21 (SPSS, Chicago, IL, USA), and effects were considered statistically significant at *P* < 0.05.

RESULTS

Baseline characteristics

The total group of 128 subjects had a mean age of 22 ± 3 years and a mean BMI of 22 ± 2 kg/m<sup>2</sup> (Table 2). Athletes trained on average 9 ± 4 h per week. Most athletes had a light skin colour (*n* = 112), and only 16 athletes had a darker skin colour. Athletes competed in different sport disciplines, classified as mainly training outdoors (total *n* = 94): field hockey (*n* = 61), athletics (*n* = 15), soccer (*n* = 12), rowing (*n* = 4), cycling (*n* = 1) and bobsleighting (*n* = 1) or indoors (total *n* = 34): volleyball (*n* = 26), synchronized swimming (*n* = 5) and judo (*n* = 3).

On average, baseline characteristics of the athletes participating in the dose–response study did not differ from those of the total group, nor did the three supplemental groups differ from each other, except for gender distribution. The sufficient group included only female indoor athletes (Table 2).

During the dose–response study, 12 participants withdrew after T0, another 13 after T3 and 7 participants withdrew after T6. A total of 40 athletes completed all measurements, of which 11 subjects in the 400 IU/day, 7 in the 1100 IU/day, 12 in the 2200 IU/day group and 10 in the sufficient group; please see Figure 2 for participant flow chart.

Baseline serum 25(OH)D

Baseline mean total 25(OH)D concentration was 64 ± 26 nmol/l in all athletes and 64 ± 21 nmol/l in the athletes who completed all measurements (*n* = 40). Of the total group, 43 athletes had a deficient (< 50 nmol/l), 46 athletes had an insufficient

Table 2. Baseline characteristics

	Total ( <i>n</i> = 128)	400 IU/day ( <i>n</i> = 31)	1100 IU/day ( <i>n</i> = 29)	2200 IU/day ( <i>n</i> = 29)	Sufficient ( <i>n</i> = 13)
Age (years)	22 ± 3	21 ± 3	22 ± 3	21 ± 3	23 ± 4
Gender (M/F)	58/70	18/13	18/11	18/11	0/13
Body weight (kg)	70 ± 8.8	71 ± 10	73 ± 10	70 ± 7	74 ± 6
Height (m)	1.78 ± 0.09	1.80 ± 0.09	1.79 ± 0.09	1.77 ± 0.09	1.81 ± 0.06
BMI (kg/m <sup>2</sup> )	22 ± 2	22 ± 2	22 ± 2	22 ± 2	23 ± 2
Indoor/outdoor (N)	34/94	6/25	6/23	2/27	13/0
Training hours per week (h:min)	8:53 ± 3:39	7:26 ± 2:42	8:08 ± 2:37	7:51 ± 2:38	7:51 ± 2:38
Self-reported sunlight exposure (scores 1–108)	13.0 ± 6.3	12.1 ± 5.8	13.6 ± 7.2	13.7 ± 6.4	12.0 ± 5.9
Skin colour (light versus darker)	112/16	27/4	24/5	25/4	12/1

Abbreviation: BMI, Body mass index. Data represent means ± s.d. The sufficient group included only female and indoor athletes and showed a higher average training load. Furthermore, no differences between groups were found by one-way ANOVA.

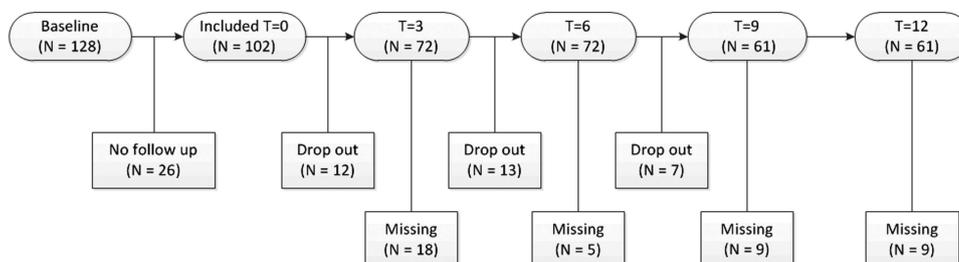


Figure 2. Participant flow chart.

(50–75 nmol/l) and 39 athletes had a sufficient (>75 nmol/l) baseline total 25(OH)D concentration. This means that 70% of all athletes showed an insufficient or a deficient total 25(OH)D concentration at baseline (the end of the winter season). A higher percentage of male athletes was vitamin D deficient or insufficient as compared with female athletes (average 25(OH)D for men and women 46 ± 19 and 78 ± 21 nmol/l, respectively;  $P < 0.01$ ).

#### Serum 25(OH)D over time

Overall, the three vitamin D dosage groups showed different effects over time (time × treatment interaction  $P < 0.01$ ), as can be seen in Table 3.

At 3 months, serum total 25(OH)D concentration was 80 ± 17 for the 400 IU/day group, 79 ± 18 nmol/l in the 1100 IU/day group, 94 ± 19 nmol/l in the 2200 IU/day group and 100 ± 22 nmol/l for the sufficient group. The increases from baseline were not different between supplementation groups ( $P > 0.05$ ). However, all three supplemental groups increased total 25(OH)D concentration more compared with the sufficient group ( $P = 0.049$  for the 400 IU/day group,  $P = 0.01$  for the 1100 IU/day group and  $P < 0.01$  for the 2200 IU/day group). In the 2200 IU/day group, a sufficient total 25(OH)D concentration was achieved in 85% of the athletes within 3 months. A sufficient total 25(OH)D concentration was achieved in 50 and 57% of the athletes within the first 3 months in the 1100 and 400 IU/day group, respectively.

At 6 months, in late summer, peak total 25(OH)D concentrations were achieved in all four groups. The 2200 IU/day group showed the highest mean of 144 ± 33 nmol/l, which was significantly different from the 400 ( $P < 0.01$ ) and 1100 ( $P = 0.03$ ) IU/day groups. The sufficient group had a mean total 25(OH)D concentration of 129 ± 32 nmol/l, the 400 IU/day group had a mean total 25(OH)D concentration of 111 ± 31 nmol/l and the 1100 IU/day group had a mean total 25(OH)D concentration of 119 ± 27 nmol/l. In the 1100 and 2200 IU/day and in the sufficient group, all athletes had total 25(OH)D concentrations above 75 nmol/l. In the 400 IU/day group, 86% of the athletes achieved a sufficient total 25(OH)D concentration.

At 12 months, all groups that received vitamin D supplements had higher total 25(OH)D concentrations as compared with baseline ( $P < 0.01$ ). The difference was more pronounced in the 2200 IU/day group (+50 ± 27 nmol/l) than in both the sufficient group (+4 ± 17 nmol/l;  $P < 0.01$ ) or the 1100 IU/day group (+25 ± 23 nmol/l;  $P = 0.049$ ), whereas the difference tended to be higher compared with the 400 IU/day group (+28 ± 24 nmol/l;  $P = 0.06$ ). The 2200 IU/day group had a total 25(OH)D concentration of 100 ± 27 nmol/l, the 1100 had 76 ± 29 nmol/l, the 400 IU/day group had 81 ± 26 nmol/l and the sufficient group had a total 25(OH)D concentration of 96 ± 22 nmol/l. After 12 months of supplementation, 80% of the athletes in the 2200 IU/day group achieved a sufficient total 25(OH)D concentration. In the 1100 IU/day group, only 43% of the athletes achieved a sufficient total 25(OH)D concentration, whereas in the 400 IU/day group this percentage was 63%.

#### Vitamin D intake

The average baseline dietary vitamin D intake was 4.2 ± 2.6 mcg/day (that is, 168 ± 104 IU/day). Dietary vitamin D intake did not affect total 25(OH)D concentration ( $P = 1.00$ ). Dietary vitamin D intake did not significantly change over time ( $P = 0.28$ ).

#### Sun exposure

Self-reported sun exposure scores varied from 0 points in winter till 84 points in summer ( $T = 6$  months). Scores only varied at 3 months between groups: the 400 IU/day group showed a higher score compared with the 1100 IU/day group (56 ± 10 versus 48 ± 8 points;  $P = 0.02$ ) and compared with the sufficient group (45 ± 7 points;  $P < 0.01$ ) but not with the 2.220 IU/day group (53 ± 9 points). No group differences were observed at 6 months.

#### DISCUSSION

The aims of the current study were first to assess the prevalence of vitamin D deficiency in highly trained athletes and second to identify the dosage of vitamin D<sub>3</sub> supplementation required to

**Table 3.** Total 25(OH)D concentrations in athletes receiving 400; 1100 or 2200 IU vitamin D<sub>3</sub> per day

	March T = 0	June T = 3	September T = 6	December T = 9	March T = 12
<b>A</b>					
Sufficient group	95 ± 12	100 ± 22	129 ± 32	102 ± 24	96 ± 22
N	13	12	11	11	11
400 IU/day	50 ± 16 <sup>a</sup>	80 ± 17 <sup>a</sup>	111 ± 31 <sup>b</sup>	85 ± 22 <sup>c,b</sup>	81 ± 26 <sup>c</sup>
N	31	23	22	19	16
1100 IU/day	49 ± 16 <sup>a</sup>	79 ± 18 <sup>a</sup>	119 ± 27 <sup>c,b</sup>	85 ± 25 <sup>c,b</sup>	76 ± 29 <sup>c,b</sup>
N	29	18	16	12	14
2200 IU/day	50 ± 15 <sup>a</sup>	94 ± 19	144 ± 33 <sup>c</sup>	120 ± 28 <sup>c</sup>	100 ± 27 <sup>c</sup>
N	29	19	23	19	20
<b>B</b>					
Sufficient group	—	5 ± 22	37 ± 30 <sup>b</sup>	10 ± 20 <sup>b</sup>	4 ± 17 <sup>b</sup>
N	—	12	11	11	11
400 IU/day	—	27 ± 22 <sup>a</sup>	56 ± 30 <sup>b</sup>	32 ± 22 <sup>b</sup>	28 ± 24
N	—	23	22	19	16
1100 IU/day	—	33 ± 19 <sup>a</sup>	70 ± 30 <sup>b</sup>	37 ± 24 <sup>b</sup>	25 ± 23 <sup>b</sup>
N	—	18	16	12	14
2200 IU/day	—	42 ± 16 <sup>a</sup>	94 ± 30 <sup>a</sup>	67 ± 27 <sup>a</sup>	50 ± 27 <sup>a</sup>
N	—	19	23	19	20

<sup>a</sup>Significantly different from the sufficient group at the same time point. <sup>b</sup>Significantly different from 2200 IU/day at the same time point. <sup>c</sup>Significantly different from T0. Data represent means ± s.d. Part A represents the average 25(OH)D concentration for each time point. Linear mixed models were applied to compare mean 25(OH)D levels between groups. Part B represents the change in 25(OH)D concentrations from baseline (T = 0). Changes over time are analysed by linear mixed models with correction for baseline 25(OH)D concentrations.

achieve and maintain a sufficient total 25(OH)D concentration throughout the year. We observed that only 30% of the highly trained Dutch athletes had a sufficient total 25(OH)D concentration ( $> 75$  nmol/l) at the end of the winter. A considerable 34% of the athletes showed a deficient total 25(OH)D concentration ( $< 50$  nmol/l), and 30% of the athletes were vitamin D insufficient (50–75 nmol/l). This observation is in line with other studies showing a prevalence of vitamin D deficiency ranging from 40 to 60%<sup>2–4</sup> and sufficient total 25(OH)D concentrations in ~44% of the athletes.<sup>28–30</sup> Supplementing athletes with 2200 IU/day was the most effective dosage to achieve and maintain a sufficient total 25(OH)D concentration throughout the year.

The current study defined vitamin D deficiency as serum total 25(OH)D concentrations below 50 nmol/l, based on data considering bone health and calcium metabolism.<sup>1</sup> Many other studies use the same definition,<sup>2,30,31</sup> whereas other researchers consider total 25(OH)D concentrations below 25 or 30 nmol/l as vitamin D deficiency.<sup>4,13</sup> If the current study would have interpreted the data according to the latter definition, only 6% of the athletes would be vitamin D deficient at baseline. The classification of vitamin D sufficiency is also under debate.<sup>28</sup> We chose to define total 25(OH)D concentrations above 75 nmol/l as sufficient, as these values are commonly used in athletic populations<sup>2,29,32</sup> and are considered to have a positive effect on training ability and sports performance.<sup>7–9</sup> Using this cutoff, 70% of the athletes had an insufficient total 25(OH)D concentration.

Cashman and colleagues calculated that ~400 IU/day of vitamin D would result in only 50% of the population reaching 25(OH)D concentrations of  $> 50$  nmol/l. According to their study, dosages of at least 800 IU/day were needed to ensure that 50% of the population would maintain  $> 80$  nmol/l 25(OH)D, and 97.5% of the adult population would maintain total 25(OH)D concentrations of  $> 50$  nmol/l during winter.<sup>15,33,34</sup> Thus, vitamin D intakes that are described to maintain adequate total 25(OH)D concentrations may not suffice to correct a low status.<sup>15,33,34</sup> In the present study, all vitamin D dosages (400; 1100; 2200 IU/day) increased total 25(OH)D concentrations from baseline to 12 months. Supplements containing 2200 IU/day were shown to be the most effective in preventing vitamin D deficiency throughout the year, resulting in 85% of the athletes achieving a sufficient total 25(OH)D concentrations after 3 months and 80% maintaining a sufficient total 25(OH)D concentration after 12 months. If the current study would have used a total 25(OH)D concentration below 50 nmol/l for selecting the dosage to achieve and maintain sufficient 25(OH)D levels, a dosage of 400 IU/day would be enough for all athletes to increase a low 25(OH)D concentration to  $> 50$  nmol/l within 3 months. After 12 months, 89% of the athletes in the 400 IU/day group were able to increase 25(OH)D concentration above 50 nmol/l, in the 1100 IU/day group this was 79% and in the 2200 IU/day group this was 100%.

The results of the current dose–response study show that, in four athletes randomized to take supplements with 2200 IU/day, total 25(OH)D concentrations dropped or remained below 75 nmol/l at 12 months (ranging from 43 to 73 nmol/l). Of those four athletes, two athletes had their supplemental dose reset to 400 IU/day after reaching a total 25(OH)D concentration above 125 nmol/l. The two other athletes showed poor compliance by taking  $< 75\%$  of the provided supplements. Therefore, we presume that daily intake of 2200 IU/day would be high enough for all athletes to reach and maintain a sufficient total 25(OH)D concentration during the course of a year. In all subjects receiving 400 IU/day from 6 months onwards, only 10% dropped their total 25(OH)D concentration under 75 nmol/l at 12 months. This shows that supplementation with 400 IU/day is adequate to maintain a sufficient total 25(OH)D concentration once serum total 25(OH)D concentrations rise above 125 nmol/l. Therefore, it is unnecessary to take a high dose of 2200 IU/day throughout the whole year.

Exceedingly high total 25(OH)D concentrations are considered as detrimental to health.<sup>35,36</sup> Upon starting the study, literature suggested that the safe upper limit of 25(OH)D was 125 nmol/l.<sup>1</sup> To keep total 25(OH)D concentrations under this upper limit, subjects with total 25(OH)D concentrations above 125 nmol/l had their dosage adjusted to a maintenance dose of 400 IU/day. Currently, total 25(OH)D concentrations up to 220–250 nmol/l are generally considered as safe.<sup>13,37</sup> If the current study had used an upper level of 250 nmol/l instead of 125 nmol/l, more athletes would have taken 2200 or 1100 IU/day during the entire intervention period. As a consequence, the percentage of athletes with a sufficient total 25(OH)D concentration after 12 months would likely have been higher in the 1100 and 2200 IU/day groups. Along with increasing the safe upper level of 25(OH)D, the upper daily intake level was also newly set to 4000 IU/day.<sup>38</sup> However, the present study supports that long-term use of such high amounts is not necessary to achieve and maintain total 25(OH)D concentrations above 75 nmol/l in healthy young athletes.

The current dose–response study was conducted in a group of 102 young, highly-trained, healthy male and female athletes. This study population is an underrepresented group in current dose–response studies, as most studies are conducted in older populations.<sup>14–16</sup> However, the lifestyle and characteristics of athletes inhibit the direct translation from other study populations, as age might affect sun exposure behaviour and/or the ability of the skin to produce vitamin D.<sup>39</sup> Despite the higher dietary vitamin D intake compared with the average Dutch population ( $3.2 \pm 2.2$  mcg/day),<sup>40</sup> athletes are at high risk of vitamin D deficiency in the winter, as shown in the current study. This vitamin D deficiency might hinder optimal sports performance. Therefore, it is important to measure total 25(OH)D concentration in athletes at the end of the winter and recommend athletes with a low total 25(OH)D concentration to take vitamin D supplements.

One of the strengths of the current dose–response study is the large sample size of 102 male and female athletes. Elite athletes are generally hard to reach as research participants and are therefore an underrepresented group in current literature. The number of elite athletes in the current study exceeds most of the previous dose–response studies. In addition, the follow-up period of 12 months allowed us to assess the effect of supplements throughout all seasons. However, the large group of athletes and the long follow-up period resulted in a relatively high number of drop-outs and missing values (Figure 2). Another strength of the current dose–response study is the inclusion of the self-reported sunlight exposure. Although an objective measure of sunlight exposure (for example a dosimeter) would possibly show a more precise estimate of the exposure to UVB radiation, we were able to assess factors as sun bed use, time spent outside and clothing habits. The development of a standardized sunlight questionnaire or the use of a dosimeter would be valuable for studies focusing on vitamin D intake and/or total 25(OH)D concentrations. Also, data on the effect of vitamin D supplements on athletic performance and general agreement on the definitions for vitamin D deficiency, insufficiency and sufficiency would be valuable for future studies.

## CONCLUSION

Vitamin D deficiency is highly prevalent among Dutch elite athletes. Athletes with a deficient or an insufficient total 25(OH)D concentration can achieve a sufficient total 25(OH)D concentration within 3 months by taking 2200 IU/day. After reaching a high total 25(OH)D concentration ( $> 125$  nmol/l), vitamin D supplementation of 400 IU/day is adequate to maintain a sufficient total 25(OH)D concentration.

## CONFLICT OF INTEREST

The project is funded by DSM, Food Specialties (Delft, The Netherlands) and the Dutch Olympic Committee (NOC)\*Dutch Sports Federation (NSF) (Arnhem, The Netherlands). EMP Backx, M Tieland, M Mensink, LJ van Loon and CPMG de Groot have no conflicts of interest. K Maase is a researcher with Unit Elite Sports, Netherlands Olympic Committee\*Netherlands Sports Confederation (NOC\*NSF). AK Kies is a researcher with DSM Food Specialties.

## ACKNOWLEDGEMENTS

We thank all the students and Lucy Okma for assisting during the tests.

## REFERENCES

- Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. *J Clin Endocrinol Metab* 2011; **96**: 53–58.
- Allison RJ, Close GL, Farooq A, Riding NR, Salah O, Hamilton B et al. Severely vitamin D-deficient athletes present smaller hearts than sufficient athletes. *Eur J Prev Cardiol* 2014; **22**: 535–542.
- Close GL, Leckey J, Patterson M, Bradley W, Owens DJ, Fraser WD et al. The effects of vitamin D(3) supplementation on serum total 25[OH]D concentration and physical performance: a randomised dose–response study. *Br J Sports Med* 2013; **47**: 692–696.
- Ducher G, Kukuljan S, Hill B, Garnham AP, Nowson CA, Kimlin MG et al. Vitamin D status and musculoskeletal health in adolescent male ballet dancers a pilot study. *J Dance Med Sci* 2011; **15**: 99–107.
- Mowe M, Haug E, Bohmer T. Low serum calcidiol concentration in older adults with reduced muscular function. *J Am Geriatr Soc* 1999; **47**: 220–226.
- Salles J, Chanet A, Giraudet C, Patrac V, Pierre P, Jourdan M et al. 1,25(OH) 2-vitamin D3 enhances the stimulating effect of leucine and insulin on protein synthesis rate through Akt/PKB and mTOR mediated pathways in murine C2C12 skeletal myotubes. *Mol Nutr Food Res* 2013; **57**: 2137–2146.
- Close GL, Russell J, Cobley JN, Owens DJ, Wilson G, Gregson W et al. Assessment of vitamin D concentration in non-supplemented professional athletes and healthy adults during the winter months in the UK: implications for skeletal muscle function. *J Sports Sci* 2013; **31**: 344–353.
- Barker T, Schneider ED, Dixon BM, Henriksen VT, Weaver LK. Supplemental vitamin D enhances the recovery in peak isometric force shortly after intense exercise. *Nutr Metab* 2013; **10**: 69.
- Wyon MA, Koutedakis Y, Wolman R, Nevill AM, Allen N. The influence of winter vitamin D supplementation on muscle function and injury occurrence in elite ballet dancers: a controlled study. *J Sci Med Sport* 2014; **17**: 8–12.
- Holick MF. Vitamin D status: measurement, interpretation, and clinical application. *Ann Epidemiol* 2009; **19**: 73–78.
- Wyon MA, Wolman R, Nevill AM, Cloak R, Metsios GS, Gould D et al. Acute effects of vitamin D3 supplementation on muscle strength in judoka athletes: a randomized placebo-controlled, double-blind trial. *Clin J Sport Med* 2015; **26**: 279–284.
- Dubnov-Raz G, Livne N, Raz R, Cohen AH, Constantini NW. Vitamin D Supplementation and Physical Performance in Adolescent Swimmers. *Int J Sport Nutr Exerc Metab* 2015; **25**: 317–325.
- Gezondheidsraad. *Evaluation of Dietary Reference Values for Vitamin D*. The Hague Health Council of the Netherlands: Hague, 2012.
- Gallagher JC, Sai A, Templin T 2nd, Smith L. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. *Ann Intern Med* 2012; **156**: 425–437.
- Cashman KD, Wallace JM, Horgan G, Hill TR, Barnes MS, Lucey AJ et al. Estimation of the dietary requirement for vitamin D in free-living adults > = 64 y of age. *Am J Clin Nutr* 2009; **89**: 1366–1374.
- Heaney RP, Davies KM, Chen TC, Holick MF, Barger-Lux MJ. Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. *Am J Clin Nutr* 2003; **77**: 204–210.
- Halliday TM, Peterson NJ, Thomas JJ, Kleppinger K, Hollis BW, Larson-Meyer DE. Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. *Med Sci Sports Exerc* 2011; **43**: 335–343.

- Clark M, Reed DB, Crouse SF, Armstrong RB. Pre- and post-season dietary intake, body composition, and performance indices of NCAA division I female soccer players. *Int J Sport Nutr Exerc Metab* 2003; **13**: 303–319.
- Brouwer-Brolsma EM, Vaes AM, van der Zwaluw NL, van Wijngaarden JP, Swart KM, Ham AC et al. Relative importance of summer sun exposure, vitamin D intake, and genes to vitamin D status in Dutch older adults: The B-PROOF study. *J Steroid Biochem Mol Biol* 2015; e-pub ahead of print 11 August 2015; doi:10.1016/j.jsbmb.2015.08.008
- Fitzgerald JS, Peterson BJ, Wilson PB, Rhodes GS, Ingraham SJ. Vitamin D status is associated with adiposity in male ice hockey players. *Med Sci Sports Exerc* 2015; **47**: 655–661.
- Blum M, Dolnikowski G, Seyoum E, Harris SS, Booth SL, Peterson J et al. Vitamin D (3) in fat tissue. *Endocrine* 2008; **33**: 90–94.
- Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. *Lancet* 1989; **2**: 1104–1105.
- Mavroieidi A, O'Neill F, Lee PA, Darling AL, Fraser WD, Berry JL et al. Seasonal 25-hydroxyvitamin D changes in British postmenopausal women at 57 degrees N and 51 degrees N: a longitudinal study. *J Steroid Biochem Mol Biol* 2010; **121**: 459–461.
- Dawson-Hughes B, Harris SS, Palermo NJ, Ceglia L, Rasmussen H. Meal conditions affect the absorption of supplemental vitamin D3 but not the plasma 25-hydroxyvitamin D response to supplementation. *J Miner Res* 2013; **28**: 1778–1783.
- Siebelink E, Geelen A, de Vries JH. Self-reported energy intake by FFQ compared with actual energy intake to maintain body weight in 516 adults. *Br J Nutr* 2011; **106**: 274–281.
- Faul F, Erdfelder E, Lang AG, Buchner AG. \*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* 2007; **39**: 175–191.
- Cashman KD, Seamans KM, Lucey AJ, Stocklin E, Weber P, Kiely M et al. Relative effectiveness of oral 25-hydroxyvitamin D3 and vitamin D3 in raising wintertime serum 25-hydroxyvitamin D in older adults. *Am J Clin Nutr* 2012; **95**: 1350–1356.
- Farrokhfar F, Tabasinejad R, Dao D, Peterson D, Ayeni OR, Hadioonzadeh R et al. Prevalence of Vitamin D inadequacy in athletes: a systematic-review and meta-analysis. *Sports Med* 2014; **45**: 365–378.
- Constantini NW, Arieli R, Chodick G, Dubnov-Raz G. High prevalence of vitamin D insufficiency in athletes and dancers. *Clin J Sport Med* 2010; **20**: 368–371.
- Hamilton B, Grantham J, Racinais S, Chalabi H. Vitamin D deficiency is endemic in Middle Eastern sportsmen. *Public Health Nutr* 2010; **13**: 1528–1534.
- Bescos Garcia R, Rodriguez Guisado FA. Low levels of vitamin D in professional basketball players after wintertime: relationship with dietary intake of vitamin D and calcium. *Nutr Hosp* 2011; **26**: 945–951.
- Galan F, Ribas J, Sanchez-Martinez PM, Calero T, Sanchez AB, Munoz A. Serum 25-hydroxyvitamin D in early autumn to ensure vitamin D sufficiency in mid-winter in professional football players. *Clin Nutr* 2012; **31**: 132–136.
- Cashman KD, Fitzgerald AP, Kiely M, Seamans KM. A systematic review and meta-regression analysis of the vitamin D intake-serum 25-hydroxyvitamin D relationship to inform European recommendations. *Br J Nutr* 2011; **106**: 1638–1648.
- Cashman KD, Hill TR, Lucey AJ, Taylor N, Seamans KM, Muldowney S et al. Estimation of the dietary requirement for vitamin D in healthy adults. *Am J Clin Nutr* 2008; **88**: 1535–1542.
- Melamed ML, Michos ED, Post W, Astor B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 2008; **168**: 1629–1637.
- Durup D, Jorgensen HL, Christensen J, Schwarz P, Heegaard AM, Lind B. A reverse J-shaped association of all-cause mortality with serum 25-hydroxyvitamin D in general practice: the CopD study. *J Clin Endocrinol Metab* 2012; **97**: 2644–2652.
- Vieth R. Vitamin D supplementation, 25-hydroxyvitamin D concentrations, and safety. *Am J Clin Nutr* 1999; **69**: 842–856.
- EFSA Panel on Dietetic Products NaAN. Scientific opinion on the tolerable upper intake level of vitamin D. *EFSA J* 2012; **10**: 2813–2858.
- MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. *J Clin Invest* 1985; **76**: 1536–1538.
- Hulshof MO KFAM, van Rossum CTM, Buurma-Rethans EJM, Brants HAM, Drijvers JJMM et al. Resultaten van de Voedselconsumptiepeiling 2003 Zeist. 2003.