

## **Vitamin D Supplementation Improve Cognitive Function, Speed, Jump, Hormones or Hematology in athletes**

### **Material and methods**

The study will be conducted from November to January for 4 or 8 weeks. The age of participant, women and man will be between 17 to 40 year. At the beginning of the project participant will be randomly divided into: the supplemented group and placebo. Exclusion criteria included vitamin D supplementation within one month prior to the study, use of multivitamin supplements, and intestinal disorders. Additional eligibility criteria to participate in the study will be outlined as follows: a) a minimum of 8 years of training experience, b) absence of injuries in the 6 months leading up to the assessments, and d) consistent engagement in training sessions a minimum of 5 times per week over the last 6 months. All participants will be informed verbally and in writing about the procedures, possible risks and benefits of the tests and provided written consent before the commencement of the study. All participants will be informed about the study protocol and procedures and provided written informed consent before participation. All research procedures were reviewed and approved by the Bioethics Committee of the Academy of Physical Education in Katowice (approval no. KB-05/2017, December 5, 2017). The study will be conducted in accordance with the principles of the Declaration of Helsinki for medical research involving human subjects.

The experiment will lasted for 4 or 8 weeks. The participants will be advised to adhere to their usual dietary routines throughout the study and refrain from the consumption of any supplements or stimulants throughout the experiment. The experimental sessions will be conducted between 8:00 and 10:00 a.m. At every stage of the study, biochemical variables, i.e., 25(OH)D<sub>3</sub>, 1,25(OH)<sub>2</sub>D<sub>3</sub> as well as body mass and body composition, were evaluated. Additionally, all participants performed a 30-meter run, during which speed will be measured at 5, 15 and 30 meters and CMJ (Countermovement Jump) tests, cognitive function and body mass and composition.

#### *Vitamin D supplementation protocol*

The participants were randomly assigned to four group: supplementation group (S=14; S/J=12 ) or the placebo group (P=12; P/J=12 ). The supplementation groups received one softgel capsule containing olive oil and cholecalciferol (derived from lanolin) at a dose of 4000 IU. The placebo groups received an identical softgel capsule containing only olive oil. All groups consumed one capsule daily in the evening after dinner for 4 or 8 weeks. The participants will be advised to adhere to their usual dietary routines throughout the study and refrain from the consumption of any other supplements throughout the experiment.

The experimental sessions were conducted between 8:00 and 10:00 a.m. At every stage of the study, biochemical variables such as 25(OH)D, morphology, testosterone, cortisol, PTH, will be evaluated. Also body mass and body composition evaluation will be performed twice. The evaluation of body composition and adipose tissue content will be performed by electrical impedance analysis (MF-BIA) using the InBody 770 (Biospace Co., Ltd., Seoul, Korea). The measurements will be taken under laboratory conditions, according to the instructions of the manufacturer.

Also performance test explained belowe will be perform at the begining and in the end of the study: Metholdology of the tests was explain belowe:

#### *The 5m, 15m and 30m sprint test*

The running times were recorded by two pairs of dual-beam Witty Gate photocells (Microgate, Bolzano, Italy). Following the warm-up phase, participants executed two successive 30-m sprints with a 5-minute rest interval in between the trials. To avert premature activation of the starting gate, participants commenced with their leading foot positioned 0.5 m behind the initial timing gate. The best time from the two trials: at 5, 15 and 30 m, was preserved for further analysis.

#### *Countermovement Jump with Arm Swing (CMJ)*

Countermovement jump with arm swing (CMJ) performance was assessed using dual force plates (ForceDecks, VALD Performance, Australia), a validated system for quantifying vertical jump kinetics and kinematics. Before each trial, participants stood quietly on the plates for 3 seconds to determine body mass and baseline force. Participants were instructed to start from an upright standing position with feet shoulder-width apart, descend to a self-selected countermovement depth, and perform a maximal vertical jump with the use of an unrestricted arm swing. Emphasis was placed on jumping “as high and as fast as possible” with maximal intent. Each athlete performed three maximal CMJ trials, separated by 1-minute passive recovery. The trial with the highest peak power output was retained for subsequent analysis. Vertical ground reaction force data were sampled at 1000 Hz and analyzed using the manufacturer’s software. Peak power was derived from the vertical force–time curve according to the impulse–momentum approach.

#### *Corsi Block Test*

The Corsi Block-Tapping Test was administered using the Vienna Test System (Schuhfried GmbH) to assess visuospatial short-term memory and visuospatial working-memory performance. The test was performed twice: before and after the 8-week vitamin D supplementation intervention. In the computerized procedure, participants viewed a fixed irregular arrangement of spatially separated blocks displayed on the screen. During each trial, a sequence of blocks was highlighted one after another, and participants were required to reproduce the presented spatial sequence by selecting the blocks in the same order using the input device, according to standardized on-screen instructions. After familiarization trials, the task began with shorter sequences, and sequence length was progressively adjusted in accordance with the standardized CORSI procedure and built-in stopping rules. Testing was conducted under standardized environmental conditions, and the response hand was not constrained. For statistical analyses, the following CORSI outcomes were used as continuous dependent variables: block time (CORSI BT, ms), reflecting the time-related component of block-sequence responding; reverse span score (CORSI RSS), reflecting performance in the reverse-order spatial span condition; and unweighted block span (CORSI UBS), representing the span-based visuospatial memory outcome without additional weighting. These variables were selected to characterize both the temporal and span-related aspects of visuospatial memory performance before and after the supplementation period. The Vienna Test System CORSI Block-Tapping Test has demonstrated satisfactory psychometric properties, including reliability and evidence of discriminant validity in neurological samples, while independent research also supports acceptable internal consistency for Corsi span outcomes (de Paula et al., 2016; Schuhfried, 2006).

### *Statistical analysis*

Data are presented as means  $\pm$  standard deviations (SD) and 95% confidence intervals (95% CI). All continuous variables were first subjected to the Shapiro–Wilk test to assess normality of distribution (Shapiro and Wilk, 1965). Homogeneity of variances across groups will be verified using Levene's test (Levene, 1960). Because the design comprised only two time points (pre- and post-intervention), Mauchly's test of sphericity will not required—sphericity is automatically and trivially satisfied with a single within-subject factor at two levels (Maxwell and Delaney, 2004). Given that the normality and homogeneity assumptions were met for the majority of outcomes, a mixed repeated measures analysis of variance (mixed repeated measures ANOVA) will be employed as the primary inferential tool. The mixed repeated measures ANOVA simultaneously tests the main effect of Time (change across measurement points), the main effect of Group (overall between-group differences), and the Time  $\times$  Group interaction (whether the change over time differs across groups). In cases where departures from normality will detected (Shapiro–Wilk  $p < 0.05$ ), mixed repeated measures ANOVA will still applied, as it is considered robust to moderate violations of normality when group sizes are sufficient (Glass et al., 1972; Lix et al., 1996). Effect sizes for the main effects and interaction will be quantified using partial eta-squared ( $\eta^2$ ), classified as small (0.01–0.059), medium (0.06–0.137), and large ( $> 0.137$ ) (Cohen, 1988). When a significant main effect of Time or a Time  $\times$  Group interaction will detected, pairwise post-hoc comparisons (pre vs. post) will performed within each group using paired-samples t-tests. The Bonferroni correction will be applied to control the familywise error rate across the four groups; adjusted p-values are reported alongside raw p-values. Effect sizes for pairwise comparisons will expressed as Cohen's d (difference in means divided by the standard deviation of the differences), with thresholds of trivial ( $d < 0.20$ ), small (0.20–0.49), moderate (0.50–0.79), and large ( $\geq 0.80$ ). Analyses will be performed using the Statistica v. 13.1 package.