Official Title: Effects of Caffeine on Physical and Perceptual-cognitive Components of Agility in Amateur Soccer Players

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Participants

The sixteen amateur soccer players aged 18 to 35 years participated in the study. All participants were active players who regularly participated in their teams' training programs and had no current health re- strictions. They were competing in lower-tier leagues, representing clubs from the third to fifth tiers of the Slovak league and the fifth to sixth tiers of the Austrian league systems. Eligibility for participation was assessed through a pre-selection online questionnaire which collected age, level of soccer experience, health status, and recent injuries. This tool was used to assess whether the players met the requirements for participation in the study. Players with recent musculoskeletal injuries, acute illnesses, cardiovascular or neu- rological disorders, visual impairments, or significant sleep disorders were excluded from the study. Goalkeepers were also excluded from the analysis, as their movement paoerns and playing load differ significantly from those of field players. Habitual caffeine intake was assessed using a validated questionnaire adapted from Bühler et al. [1], which tracked the frequency of caffeine-containing foods and beverages. Based on these data, individual differences in habitual caffeine consumption were identified, and subjects with a habitual caffeine intake exceeding 400 mg per day were excluded from the study. Exces- sive caffeine intake can lead to negative effects that could affect athletic performance and distort study results [2]. Initially, 30 soccer players completed the questionnaire, of whom 24 subjects subsequently met all inclusion criteria. Due to non-participation in the second measurement session or injuries during preparation, the final analyzed sample was adjusted to sixteen participants. All participants were thoroughly informed about the objectives and planned proce- dures of the study and provided wrioen informed consent. The study was conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and was officially approved by the Ethics Committee of the Faculty of Physical Education and Sport, Comenius University in Bratislava (Approval No. 15/2024).

Experimental design

The study employed a randomized, double-blind, placebo-controlled crossover de- sign to investigate the acute effects of caffeine on explosive lower-body power and agility in amateur soccer players. Each participant underwent two testing sessions under differ- ent conditions: (I) caffeine and (II) placebo. The order of conditions was randomly deter- mined for each participant using an independent online generator (random.org). Simple randomization was used, without stratification or blocking. This randomization was per- formed by an investigator not involved in testing. Supplementation was administered as caffeine capsules (Scitec Nutrition Caffeine, 100 mg per capsule) or placebo capsules filled with potato starch. Participants received 300 mg of caffeine, which represented approxi- mately 3.74 mg·kg^{-1} of mean body mass. This dosage corresponded to an individual dose ranging from 3.16 to 4.44 mg·kg^{-1} of body mass. The capsules were visually identical, and neither the testers nor the participants were aware of the assigned condition. A week be- fore the first measurement, all participants underwent a familiarization session during which anthropometric data were collected, and the testing procedures were practiced. Throughout the experiment, they were required to follow the same training regimen, re- frain from consuming caffeinated products for at least 24 hours prior to testing, and avoid intense physical activity on the previous day. Diet, fluid intake, and sleep were not stand- ardized or monitored before testing.

On both test days, participants received caffeine or a placebo in the laboratory. Thirty minutes after supplementation, all participants underwent a standardized warm-up con- sisting of dynamic aerobic and activation exercises. The warm-up included one minute of jumping jacks, followed by forward and lateral lunges, three dynamic stretching exercises, and three countermovement jumps. Before the agility tests, elements of athletic drills and one trial of the planned agility test on both sides were included to ensure optimal neuro- muscular activation before the actual performance. Testing began 30 minutes after the standardized warm-up capsule ingestion and was completed within a 15-minute window to correspond to the typical onset of physiological effects of caffeine [3]. Firstly, explosive strength tests were performed under laboratory conditions. Specifically, the countermove- ment jump (CMJ) and drop jump (DJ) tests were performed. Immediately after completing these tests, the participants moved to an artificial turf field, where they underwent a series of agility tests in a fixed order: planned agility (Y-shaped planned agility test), reactive agility to a light stimulus (Y-shaped light reactive agility), and reactive agility to a live stimulus (Y-shaped live reactive agility). The order of the tests was identical in both ex- perimental conditions, meaning that participants performed the CMJ, DJ, Y-shaped planned agility, reactive light-stimulus, and reactive live-stimulus tests in a fixed or- der.The order of the tests was identical in both experimental conditions. The tests were conducted during the same part of the season (November-December) and at the same time of day to minimize the influence of circadian variation. Individual visits were separated by a seven-day interval, which provided sufficient time for recovery and ensured con- sistency in the training load of participants throughout the experiment. A schematic overview of the experiment is shown in Figure 1.

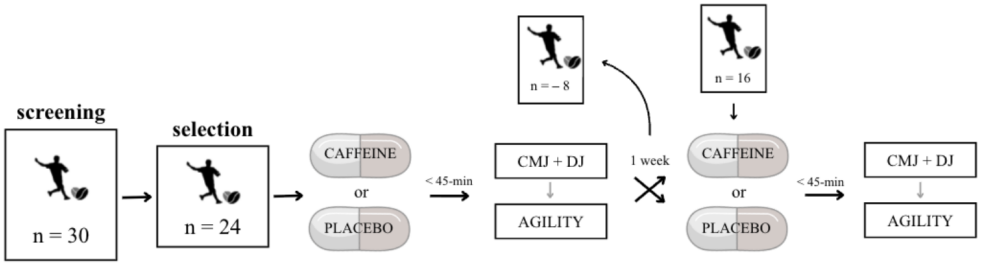


Figure 1. Schematic experimental design of study.

Vertical jump tests

The countermovement jump (CMJ) without an arm swing was implemented as a valid and reliable method for evaluating lower-limb explosive power. Participants initi- ated the movement from an upright standing position with their hands placed on their hips. They were then instructed to bend their knees to approximately 90° and jump as high as possible. This CMJ protocol is considered a standardized testing procedure to measure lower-limb explosive power in team sports [4]. Three trials were performed, with approximately 30 s of rest between aoempts. The highest jump height was recorded as the outcome of the testing procedure. The drop jump (DJ) from a height of 30 cm was employed to evaluate the reactive strength of the lower limbs. Participants initiated the test by standing on a 30 cm box with their hands positioned on their hips. They were instructed to step off the box, land with both feet simultaneously, and rapidly execute a maximal vertical jump while minimizing ground contact time (CT). Flight time (ms) and ground CT (ms) were recorded for each jump. The reactive strength index (RSI) calculated from these variables was considered as
outcome for this assessment. The RSI has been shown to be a valid and reliable indicator of explosive performance [5]. Three trials were conducted, with approximately 30 s of rest between aoempts. The jump with the highest RSI was recorded as the result of the assessment. Both jump tests were assessed using the OptoGait system (Microgate, Bolzano, Italy).

Planned and reactive agility tests

To measure the level of agility, we employed the Y-shaped setup of tests [6,7] using a Wioy GATE system with integrated Wioy SEM sensor (Microgate, Bolzano, Italy). Sub- jects had to start from 30 cm behind the starting gate and change the direction of move- ment in the area marked with cones in the middle of the whole running distance (Figure 2). There were three types of testing procedures. In the first procedure, subjects were in- structed about the COD prior to the trial. A total of four trials were performed, with two direction changes to each side. This procedure measured the level of planned agility, i.e., COD speed. The second procedure included a light sensor that triggered the green arrow stimulus with a delay of 500 ms after breaking the beam of the starting gate. The side of the stimulus was randomly assigned by the system. This procedure was finished after the system assigned two CODs to each side. The third procedure included a human stimulus [28]. A tester stood 4 m from the middle cone gate. Once the subject crossed the first cone gate 2.5 m apart from the starting line, the tester performed one of the two possible move- ments in a randomized order: 1. Step forward with right foot and change direction to the left, 2. Step forward with the left foot and change direction to the right. The subject had to evade the movement meaning they should run to the opposite final gate. Four trials con- sisting of two randomized CODs to each side were performed. In case a participant made a technical error, slipped, or chose an incorrect gate, a new repetition was permioed. In case that the light or live reactive agility time was lower than or equal to the planned agility time, the trial was considered invalid and repeated. This helped to reduce possible bias where subjects anticipated the side of COD.

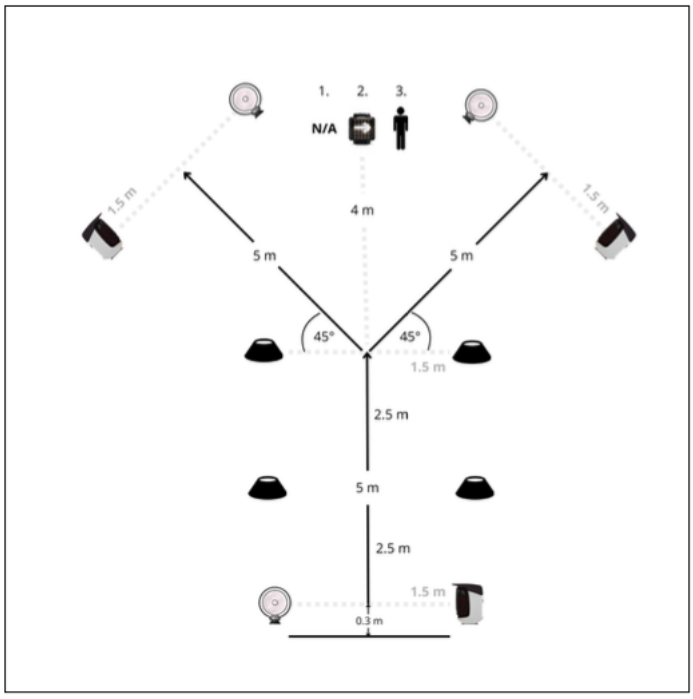


Figure 2. Setup of Y-shaped planned and reactive agility tests.

Statistical analysis

The statistical power for the sample size was computed using G*Power (Version 3.1.9.6, Institut für Experimentelle Psychologie, Düsseldorf, Germany). The power was established a priori for an effect 0.5 with a power of $1 - \beta = 0.80$ and $\alpha = 0.05$ ($n = 24$). Due to participant dropout for objective reasons the final sample ($n = 16$) provided a power of $1 - \beta = 0.60$. The Shapiro-Wilk test was used to assess the normality of the data. A paired sample t-test compared performance after placebo and caffeine ingestion. Differences between conditions were further expressed as standardized mean differences of effect size with a 95% confidence interval (95% CI) [8]. Thresholds for effect size (ES) statistics (Hedges' g) were defined as follows: <0.2, trivial; 0.2-0.6, small; 0.6-1.2, moderate; 1.2-2.0, large; and >2.0, very large [8,9]. Statistical analyses were conducted utilizing GraphPad Prism software version 10.3.1 (GraphPad Software, San Diego, CA, USA).

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