Effects of Beta-Caryophyllene Supplementation on Autonomic Regulation and Apnea Performance in Elite Divers - a Randomized Crossover Trial

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Project summary

This project recruited 15–20 healthy adult male active freedivers. Using a randomized crossover design with double-blind methodology, participants were assigned to either the β-caryophyllene trial (BCP) trial or the placebo (PLA) trial, which without β-caryophyllene. Participants consumed either 200 mg of β-caryophyllene capsules (BCP trial) or placebo capsules (PLA trial) based on pre-test results. After a 30-minute rest period and warm-up, a single maximal voluntary static breath-hold test was conducted. Breath-hold duration and physiological and salivary biochemical indicators before and after the breath-hold test were observed.

Introduction

Currently, no specific nutritional supplements have been established internationally to enhance athletic performance in freediving. Activation of the cannabinoid receptor type 2 (CB2) may represent a significant approach to further enhance freediving capabilities. Therefore, this study investigates the effects of β-caryophyllene (BCP) supplementation on static apnea performance by examining its influence on CB2, which may improve autonomic nervous system function and increase pain tolerance.

Methods

Study design

This study employed a randomized crossover design with double-blind methodology, dividing subjects into either the β-caryophyllene trial (BCP) group or the placebo trial (PLA) group without β-caryophyllene. Testing occurred during the off-season. Excel software randomly assigned participants to groups and generated unique identification numbers for each subject. The experimental sequence was determined through random allocation. Three days prior to the first formal experiment, subjects were instructed to record all dietary intake and consume identical meals during subsequent formal experiments. Each experiment commenced at 9:00 AM, with subjects required to arrive at the laboratory in a fasting state for testing.

Participants

This study plans to recruit 15–20 healthy adult males with training in freediving or long-distance swimming as subjects. According to the International Association for the Development of Apnea (AIDA) classification system, inclusion criteria are: 1. At least 3 years of training in freediving or long-distance swimming, with the highest AIDA certification level (Level 4). 2. Absence of cardiovascular or joint diseases. 3. Recovery from sports injuries such as strains or sprains for at least 3 months. Exclusion criteria are: 1. Insufficient training duration. 2. Presence of cardiovascular or joint diseases, or any conditions susceptible to exercise-induced damage. 3. Female or underage subjects. 4. Individuals allergic to β-caryophyllene. The consent form is completed after the participant has given consent. This study was conducted following the Declaration of Helsinki.

Protocol

All formal experiments are scheduled to begin at 9:00 AM, with subjects arriving at the laboratory in a fasting state. After a brief rest period, the first saliva sample is collected. Subjects are connected to a gas analyzer and heart rate monitor, and their resting metabolic rate, heart rate, and heart rate variability are recorded for 10 minutes in a supine position. Following this, subjects ingest either β-caryophyllene (BCP group) or a placebo capsule (PLA group). After a period of quiet rest, the same measurements are taken again.

Following this, subjects ingest either β-caryophyllene (BCP trial) or a placebo capsule (PLA trial). After resting quietly, a second 10-minute recording of resting energy expenditure, heart rate, heart rate variability, and a second saliva sample are collected in the supine position. Based on pre-test results, β-caryophyllene exhibited optimal efficacy 30 minutes post-administration. Thus, subjects rested quietly for 30 minutes. During this period, subjects continuously wore gas analyzers and heart rate monitors, with near-infrared spectroscopic cerebral oximetry (NIRS) sensors attached to the frontal lobe. NIRS cerebral oximetry) on the forehead to measure cerebral oxygenation. A pulse oximeter was attached to the fingertip. Subsequently, participants performed a maximal static breath-hold test following three submaximal breath-holds, replicating the protocol of Hoiland et al. (2017) (1).

β-Caryophyllene capsules and placebo

The same brand of β-caryophyllene has also been used in previous studies (2). Using dried, purified -caryophyllene powder, subjects received optimized β-caryophyllene supplementation doses based on preliminary testing results, administered via capsules.

lacebo capsules contained flour. All preparation processes were completed by non-experimental staff and provided to experimental staff through numbering to achieve double-blind conditions.

Saliva sample collection and analysis

Saliva samples were stored in a refrigerator at -80 degrees immediately after collection. For analysis, samples were thawed and centrifuged at 4000 rpm for 5 min. 500 μL of saliva samples were transferred to glass tubes for mass spectrometry analysis and Enzyme-linked immunosorbent assay (ELISA) analysis respectivelyThe analysis of saliva α-amylase concentration was conducted utilising an enzyme-linked immunosorbent assay (ELISA) with commercially available reagents (Neogen Corporation, Kentucky, USA; Salimetrics LLC, State College, PA, USA).

Statistical analysis

All data are presented as means ± standard deviations. The Shapiro–Wilk test was utilised to evaluate the normality of the data. The grip strength and number of fall on each rounds between the two trials were analyzed using paired t-tests. The saliva caffeine and α-amylase concentration and RPE were analyzed using two-way ANOVA with repeated measures. If the interaction effect (trial × time) were significant, the Bonferroni method was used to perform post hoc comparisons. Effect sizes were calculated using Cohen's d to quantify the magnitude of observed ef-fects and defined as trivial (<0.20), small (0.20–0.40), moderate (0.40–0.80), and large (>0.8), respectively. The power value of each data was conducted using G*Power 3.1.9.6 software (3)

Reference

1. Hoiland RL, Ainslie PN, Bain AR, MacLeod DB, Stembridge M, Drvis I, et al. β1-Blockade increases maximal apnea duration in elite breath-hold divers. Journal of applied physiology. 2017;122(4):899-906.
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