

Study Protocol and Statistical Analysis Plan

Study Title: Effects of Daily Supplementation of 5-HTP on Body Composition, a Randomized Investigation

Protocol Version 2

Document Date: 01/03/22

Protocol ID# 102

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PROTOCOL SIGNATURE

I confirm that I have read this protocol, and I will conduct the study as outlined herein and according to the ethical principles stated in the latest version of the Declaration of Helsinki, and the applicable laws and regulations of the federal government. I will promptly submit the protocol to the IRB for review and approval. I understand that any modification made during the course of the study must first be approved by the IRB prior to implementation except when such modification is made to remove an immediate hazard to the subject.

List of Abbreviations

| Abbreviation or Term | Definition/Explanation |
|-----------------------------|---|
| 5-HTP | 5-Hydroxytryptophan |
| PLA | Placebo |
| SSRIs | Selective serotonin reuptake inhibitors |

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Abstract

The purpose of this investigation was to determine the effects of supplementing with 100 mg daily of 5-Hydroxytryptophan (5-HTP) on indices of body composition in exercise-trained men and women.

Specific Aims:

Aim 1: Anthropometric measurements will be collected to determine if 5-HTP supplementation can induce changes in body composition.

Aim 2: Self-reported dietary intake will be tracked using MyFitnessPal to determine if daily 5-HTP supplementation influences eating behaviors.

Aim 3:

Methods: Subjects were randomized into a treatment (100 mg 5-HTP daily; CLEANMOOD™) or a placebo (maltodextrin). Body composition was assessed pre- and post-treatment after eight weeks via a multi-frequency bioelectrical impedance device (InBody® 270). Subjects were instructed to not change their training or eating habits; moreover, they were instructed to track their diet ~2-3 days per week using a mobile app (MyFitnessPal).

Hypothesis:

Compared to placebo, daily supplementation with 5-HTP will result in changes in body composition.

Background

5-Hydroxytryptophan (5-HTP) is an amino acid and intermediate metabolite of L-tryptophan. 5-HTP crosses the blood-brain barrier, stimulating serotonin (5-hydroxytryptamine) receptors in the central nervous system.[1, 2] Increasing serotonin levels may lead to changes in eating behavior, such as reduced calorie intake and increased satiety.[3, 4] When consumed orally, 70% of 5-HTP is delivered to the bloodstream. Once absorbed, 5HTP supplementation increases serotonin production and other brain chemicals such as melatonin, endorphins, dopamine, and norepinephrine. Supplementing with 5-HTP is linked to appetite suppression, improved sleep quality and decreased feelings of anxiety and depression. [2, 3, 5-8] 5-HTP has been posited to be an effective weight-loss aide due to its anorexigenic effects; however, little data exists and virtually all investigations have studied overweight or obese subjects. Cangiano et al. showed that 5-HTP supplementation influenced energy intake in non-insulin dependent diabetic subjects. Subjects receiving 5-HTP consumed less energy via decreased consumption of fats and carbohydrates resulting in weight loss.[5] Another study observed the effects of a 5-HTP containing supplement on body composition in overweight females. Subjects were instructed to reduce their energy intake moderately. Following 4-weeks of 5-HTP supplementation, significant reductions in BMI, arm circumference and hip circumference were observed. Increased feelings of satiety were greater in the supplement group when compared to placebo.[8] Ioannou and Williams examined the effects of 5-HTP intake on brain activity as it relates to food sensitivity in healthy individuals.[6] Subjects were randomly assigned to a 5-HTP or vitamin C supplement group. This investigation discovered that 5-HTP intake influences areas of the brain related to macronutrient selection and increased awareness of the body's internal physiological needs. The vitamin C group was more responsive to high caloric and fatty foods compared to the 5-HTP group. This study suggests that 5-HTP intake can influence macronutrient intake as well as modulate eating behaviors. Presently, there

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are no studies that have assessed the effects of 5-HTP in exercise-trained individuals. Therefore, the purpose of this investigation was to determine the effects of supplementing with 100 mg daily of 5-hydroxytryptophan (5-HTP) on indices of body composition in exercise-trained men and women.

Study Objectives

Primary Objective

To assess changed in body composition.

Secondary objective

To assess any changes in dietary intake.

Study Methods

Study Design

This study followed a placebo (PLA)-controlled, double-blind and parallel-group design.

Study population

61 physically active, healthy, adults (>18) were volunteered for this study.

Inclusion-Exclusion Criteria

Inclusion Criteria:

- Healthy or free of disease
- Minimum of 1 year of regular physical activity. Regular physical activity is defined by a minimum of 150 minutes of aerobic activity or performing muscle-strengthening exercises at least 2 days a week.

Exclusion Criteria:

- Physically inactive
- Regularly used sleep aids of any kind
- Currently taking selective serotonin reuptake inhibitors (SSRIs) and/or 5-HTP containing supplements.

Discontinuation or Withdrawal from Study

The reasons a subject may discontinue or be withdrawn from the study include, but are not limited to, adverse events, non-compliance or personal request to no longer participate in study.

Randomization and Blinding

All subjects and investigators were blinded. The supplement and placebo were in similar in capsule appearance and were packed in unmarked packaging. The research team were unaware of the subject assignments until completion of the entire study.

Intervention and Placebo

The dietary supplement (100 mg of 5-hydroxytryptophan; CLEANMOOD™) was provided by NURA™ (Irvine, California USA). CLEANMOOD is an organic 5-Hydroxytryptophan (5-HTP) ingredient. The placebo consisted of maltodextrin in a capsule. Subjects were instructed to consume one capsule daily at their convenience for 8 weeks.

Data Collection Schedule

Subjects were instructed to arrive at the laboratory after a 3-hour fast. All pre and post-testing occurred between 1200 and 1700 hours.

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| Study Visit | Pre/Baseline | Post |
|--|--------------|------|
| Informed Consent | x | |
| InBody | x | x |
| Vitals | x | x |
| Supplement Instruction | x | |
| Connect MyFitnessPal account | x | |
| Asses dietary intake via MyFitnessPal | x | x |
| Asses Compliance (number of pills remaining) | | x |

Missing Data

Thirteen subjects were excluded from the data set due to lack of compliance or failure to complete the study.

Statistical Analysis

All data is presented as the mean+SD. GraphPad (Prism 8) software was used for statistical analyses. A t-test was used to determine if statistically significant differences ($p < 0.05$) existed between the delta scores of the treatment versus the placebo.

Summary of Study Data

Demographic and Baseline

The characteristics of the treatment and placebo groups were as follows (age: 5-HTP 30 ± 10 , placebo 29 ± 11 ; height cm: 5-HTP 171.0 ± 8.7 , placebo 167.7 ± 15.0 ; body mass kg: 5-HTP 72.9 ± 13.4 , placebo 69.1 ± 11.9). Subjects in this investigation were exercise-trained (years of training: 5-HTP 14 ± 10 , placebo 16 ± 11 ; hours of resistance training per week: 5-HTP 5 ± 2 , placebo 5 ± 3 ; hours of aerobic training per week: 5-HTP 5 ± 4 , placebo 6 ± 5).

FDA Annual Reporting

This study is not a FDA regulated drug or device, therefore there are no annual reporting requirements to the FDA.

Clinical Trial Data Bank

The study will be registered on <http://clinicaltrials.gov> and the NCI CTRP (Clinical Trials Reporting Program) by the Clinical Trials Office.

References

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