

## **Black Cumin Seed Oil Plus Fish Oil Combination Modulates Gut-Immune-Axis**

**Shawn M Talbott<sup>1,2\*</sup> and Julie A Talbott<sup>2</sup>**

<sup>1</sup>*Amare Global, Irvine, CA, USA*

<sup>2</sup>*3 Waves Wellness, Plymouth, MA, USA*

**\*Corresponding Author:** Shawn M Talbott, Research and Development, Amare Global and 3 Waves Wellness, USA.

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### **Abstract**

We evaluated the effects of a dietary supplement combination on upper-respiratory tract complaints (URTCs) and psychological mood state. Thirty-five male (19) and female (16) runners consumed an oil blend (500 mg of black cumin seed oil plus 1,500 mg fish oil) or a placebo daily during the 4 week supplementation period (3 weeks before and 1 week following a marathon or half-marathon competition). We collected subjective and objective measures before and after supplementation. Subjects completed the profile of mood state (POMS) psychological assessment and a health log questionnaire measuring health status and URTCs (subjective measures), as well as provided saliva samples and fecal samples for measurement of cortisol and microbiome balance, respectively (objective measures). Subjects in the supplementation group (black seed/fish oil blend) reported significantly fewer upper-respiratory tract complaints (URTCs) and better overall well-being, as well as higher omega3 blood levels, lower cortisol and superior microbiome indices, compared to placebo. These results suggest that the combination of black cumin seed oil plus fish oil may improve immune system vigilance and overall well-being following the stress of endurance training and competition, possibly via improvements in the microbiome and Gut-Immune-Axis.

**Keywords:** *Upper-Respiratory Tract Complaints (URTCs); Profile of Mood State (POMS); Black Cumin Seed Oil; Fish Oil; Gut-Immune-Axis*

### **Introduction**

The immune system is traditionally thought of as the body's primary defense against external pathogens such as viruses. Increasingly, research is demonstrating an expanded role of the immune system as both a "shield" against viruses and also as a "communication organ" via its contribution to psychological mood state and overall well-being.

Numerous studies have shown the close links between psychological factors such as stress, sleep deprivation, and immune suppression leading to poor vaccine responses and increased upper-respiratory tract infections (URTIs) and non-infection upper-respiratory tract complaints (URTCs). Athletes in particular, because of the mental and physical stress of their heavy exercise and intense training regimen, tend to have increased susceptibility to URTI and URTC [1-4]. Exercise has been demonstrated both a physical and psychological stressor that can have significant negative impacts on immune system components such as neutrophils, natural killer cells, T cells and B cells [5-7]. The one-to-two-week period following intense endurance competition represents a particularly susceptible period of time during which athletes often present with a range of upper-respiratory tract complaints (URTC), such as cough, stuffy nose, sore throat, headaches and others. These complaints may be related to temporary elevations in hormones such as cortisol and inflammatory cytokines that coordinate the stress response [8,9]. The chronic impact of continuous heavy endurance training (without adequate recovery) is overtraining or overreaching syndrome and eventually athlete burnout, which is characterized by a weakened immune system, increased risk of URTIs/URTCs, and negative outcomes for psychological mood state (e.g. higher stress, poor sleep quality, increased risk for depression, among others).

Like psychological stress, exercise stress is known to suppress immune system vigilance and increase susceptibility to many disease states, including URTIs/URTCs [10]. Habitual exercise is widely regarded as a buffer against many detrimental health effects of stress, but chronic overload (in the absence of adequate recovery), which can be common in recreational athletes building up for an endurance competition such as a half-marathon or marathon, can also lead to psychological stress deteriorations in mood state [11]. Individual ability to cope with daily stressors is known to influence the immune response to exercise - with over-stress associated with reductions in immune cell populations, lowered antibody production and altered cytokine response [12,13].

A wide range of dietary supplements have shown varying levels of effectiveness in balancing the relationship between exercise stress and immune vigilance. For example, various vitamins and minerals (C, D, zinc), yeast beta-glucans and mushroom alpha-glucans have shown promising benefits in endurance runners [14-22] via their ability to enhance the innate immune response, helping to prime immune system function during and following various forms of chronic stress.

Omega-3 fatty acids are well-described in the scientific literature for their anti-inflammatory effects and thus provide benefits across a wide range of health parameters, including supporting the immune system [23,24]. Because of the close relationship between inflammatory balance and both physical and mental well-being, omega-3 fatty acids may help to support quality of life parameters, particularly after stress, which is known to disrupt both inflammatory signaling and psychological mood state.

Black cumin seed oil (*Nigella sativa*) oil is one of the most revered herbal medicines in Middle-Eastern and Ayurvedic traditional medicine - buried with Egyptian pharaohs to aid in the afterlife journey and mentioned in the Bible and the Koran where they are referred to as “the blessing seed created by God” to relieve difficult medical conditions.

Research on black cumin seed oil has spanned preclinical, animal, and human clinical studies, with many of the therapeutic properties of the plant attributed to its essential oil constituent, thymoquinone (TQ), which may have immunomodulatory effects on macrophages, lymphocytes, macrophages, natural killer (NK) cells, and a variety of immune-related cytokines (IFN, IL-2, TNF-alpha, IL-6 and others).

Omega-3 fatty acids (EPA, eicosapentaenoic acid and DHA, docosahexaenoic acid) have immune-regulating and inflammation-balancing functions. Omega-3s can also influence the gut microbiome by modulating the type and abundance of gut microbes and modulating the levels of proinflammatory mediators, such as endotoxins and lipopolysaccharides [23,24]. Omega-3s could provide benefits for the gut microbiome in a variety of ways, such as limiting the growth of “bad” bacteria (such as *Enterobacteria*); enhancing the growth of “good” bacteria (such as *Bifidobacteria*); and modulating metabolic endotoxemia and the associated immune/inflammatory response [23]. Omega-3 supplementation has been shown to alleviate dysbiosis of the gut microbiome dysbiosis by increasing the growth and relative abundance of “good” bacterial species such as *Lactobacillus*, *Bifidobacterium*, as well as various butyric acid-producing species [23].

Stress-related gut dysbiosis and immune alterations can represent significant and meaningful challenges for not only the exercise performance of athletes, but also their overall health and well-being. In this study, we used the “exercise stress” of endurance running to challenge gut microbiome resilience and temporarily suppress immune vigilance. We have used this exercise stress model in a number of previous investigations of immune system function [8] - and apply it here to elucidate the potential benefits of black cumin seed oil and fish oil in supporting the Gut-Immune-Axis.

### Materials and Methods

This study was done in accordance with the Helsinki Declaration, for clinical research involving human subjects and was reviewed and approved by an external advisory board (WCG-IRB, Puyallup, WA; Protocol #20202070).

The objective of this study was to investigate the effects of dietary supplementation for one-month with a combination of black seed and fish oils. Our hypothesis was that this oil combination may be immunomodulatory for improving immune system vigilance

and psychological stress. We recruited 40 healthy volunteers to receive the Supplement (oil blend) or a Placebo (maltodextrin). The supplement provided 500 mg of black cumin seed oil as ThymoQuin (3% thymoquinone, TriNutra, Israel) and 1,500 mg fish oil providing 1,200 mg Omega3 fatty acids (VivoMega, GC Rieber, Norway).

### Subjects

We recruited healthy, fit, recreational runners who were experienced in training and competing for half-marathon to marathon-distance events. Our subject pool completing all phases of baseline pre-supplementation measurements, training, competition, and final post-supplementation measurements included 35 subjects (Table 1). Three subjects in the Supplement group and two subjects in the Placebo group were lost to follow up (did not complete all measures). There were no adverse events reported for either group.

Group	Average Age	Men	Women	Total
Supplement	38 ± 4	9	8	17
Placebo	36 ± 5	10	8	18

*Table 1: Subject demographics.*

### Health log

Subjects completed a physical health questionnaire [8] at baseline (pre-supplementation) and 4-weeks (post-supplementation). The health log was a daily health perception log containing questions related to overall health status and specific upper-respiratory tract complaints (URTCs). The URTC-related symptoms measured included nasal congestion, runny nose, sore throat, sneezing, cough, fatigue, headache, general malaise and body aches. Reported symptoms were totaled for each assessment period.

### Mood assessment

Changes in psychological mood state were assessed using the research-validated Profile of Mood States (POMS) questionnaire to measure 6 primary psychological factors (tension, depression, anger, fatigue, vigor and confusion) plus the combined global mood state as an indication of subjective well-being. The POMS methodology has been used in ~3,000 studies, and its validity is well established [25]. The POMS profile uses 65 adjective-based intensity scales scored on a 0 - 4 hedonic scale (e.g. “not at all” to “extremely”). The 65 adjective responses are categorized into the 6 mood factors (tension, depression, anger, fatigue, vigor, or confusion), tabulated, scored and analyzed. The output of the POMS questionnaire is an assessment of the positive and negative moods of each subject at baseline and post-supplementation.

### Salivary cortisol

Cortisol is the major glucocorticosteroid stress hormone produced in the adrenal cortex and is actively involved in regulating many aspects of metabolism related to sports performance, including blood pressure, anti-inflammatory function, gluconeogenesis and immune function. Cortisol production has a circadian rhythm, with peak levels in the early morning and nadir levels at night during deep sleep. Levels rise independently of circadian rhythm in response to stress. Salivary cortisol represents a simple and accurate method for assessing unbound active cortisol levels as an index of overall stress exposure. Each subject provided “first morning” saliva samples (upon awakening) for analysis of free cortisol at baseline (pre-supplementation) and 4-weeks (post-supplementation).

### Microbiome assessment

Volunteers were provided with a take-home kit to obtain fecal samples in the privacy of their home for analysis. The kit included detailed instructions and postage paid packaging for return directly to the lab. Each kit was numerically coded so that samples were blinded to the lab. Microbiome analysis of fecal samples was carried out using the complete BiomeTracker system (Wasatch Scientific, Murray, UT). Briefly, fecal samples were obtained by nylon swab and placed into preservative binding buffer to lock the composition of bacteria in place. DNA was then purified using DNA columns and ~20 ng of DNA from each sample was added to the reaction mixtures. Samples were processed on an ABI 7500 Fast (Applied Biosystems) instrument in duplicate. A “microbiome composite score” was generated as an overall average of many different aspects of microbiome balance, including *Bifidobacterium*, *Lactobacillus*, *Akkermansia*, *S. Thermophilus*, *Firmicutes/Bacteroidetes* (F/B) ratio and others.

### Blood omega-3 index

Blood levels of omega-3 fatty acids were assessed using a self-collected at-home fingerstick blood sample (OmegaQuant Analytics, Sioux Falls, SD). Subjects received all supplies and instructions to apply a drop of blood to the collection card and mail sample to the laboratory in a pre-paid envelope.

### Data management and analysis

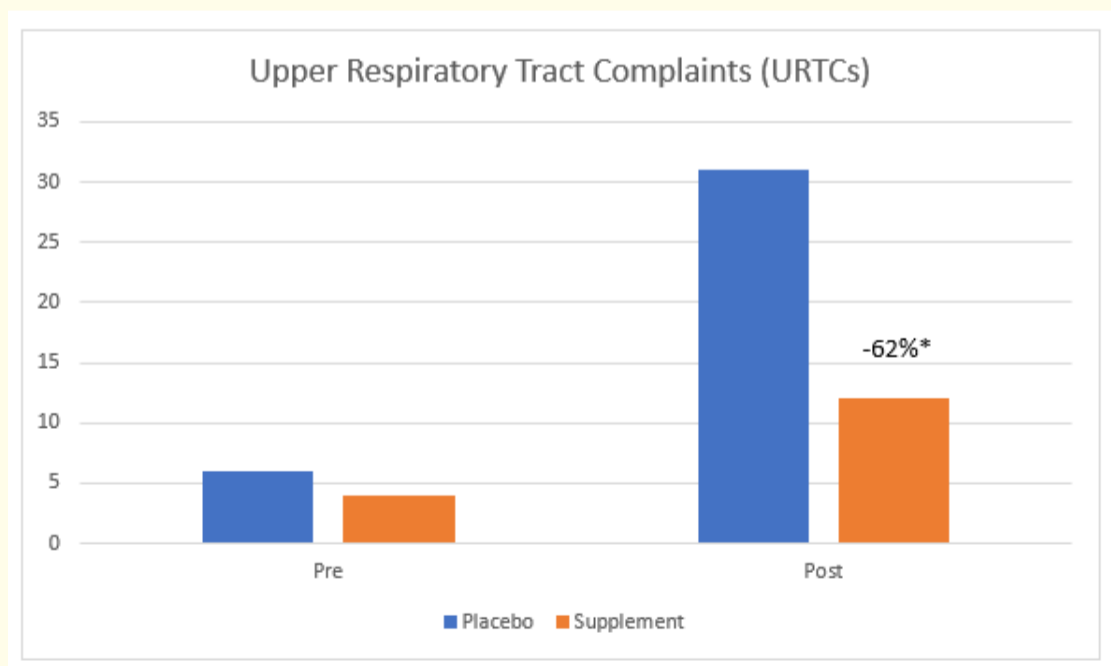
All questionnaires were hand-delivered or mailed to a central location and transcribed to a central database. Subjects who did not complete the questionnaires or who submitted incomplete questionnaires were dropped from the study and not included in the study analysis (6 subjects: 4 from the Supplement group and 2 from the Placebo group). Data were identified by subject number and examined for accuracy and completeness. Tabulated data were analyzed with JMP 14.0 (JMP Statistical Discovery, Cary, NC) using standard parametric paired t tests, and significance was assessed with a 2-tailed alpha level set at 0.05. Data are presented as average values for each group (Placebo and Supplement) before and after supplementation.

## Results

Following 4 weeks of supplementation with the oil blend, (3 weeks before and 1 week following an intense endurance run), we observed the following differences between the supplement and placebo groups.

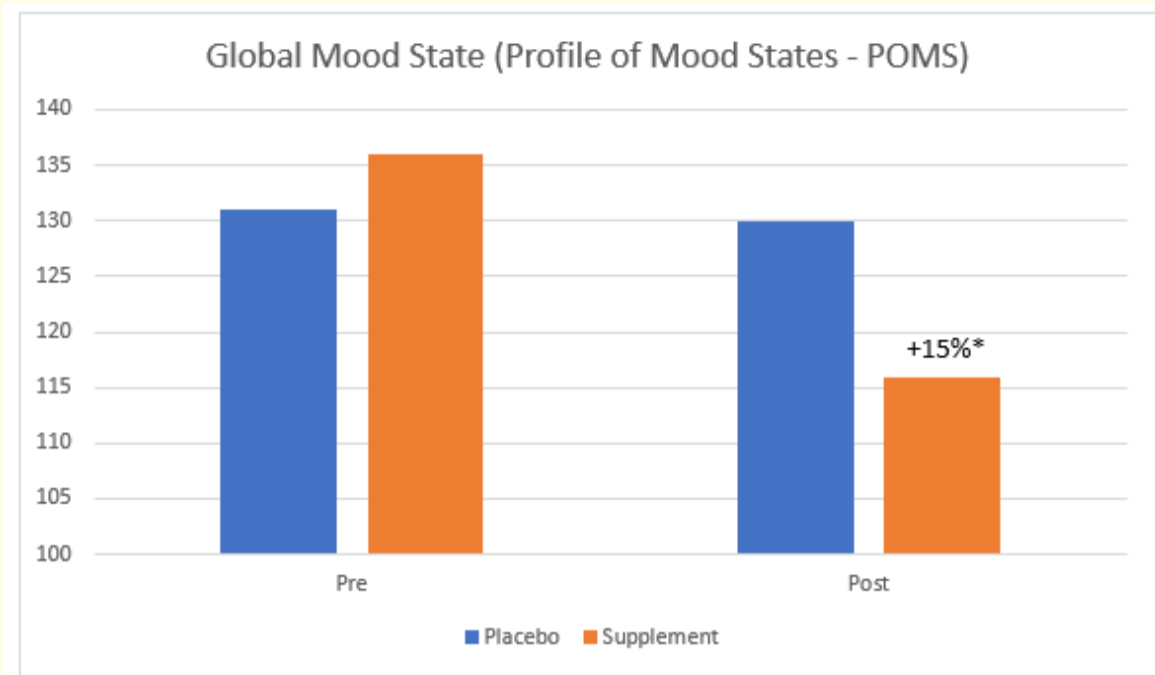
### Subjective measures

As expected, both groups reported dramatically more self-reported upper-respiratory tract complaints (URTCs) following the endurance run compared with before (Figure 1). However, URTCs, including the total number of symptoms reported such as cough, sore throat, sniffles, stuffiness, etc. were 62% lower in the supplement group compared to placebo (Figure 1).



**Figure 1:** Subjects in the supplement group had significantly fewer self-reported upper respiratory tract complaints (URTCs) compared to placebo. (\*Significantly different from post-supplementation placebo value,  $p < 0.05$ ).

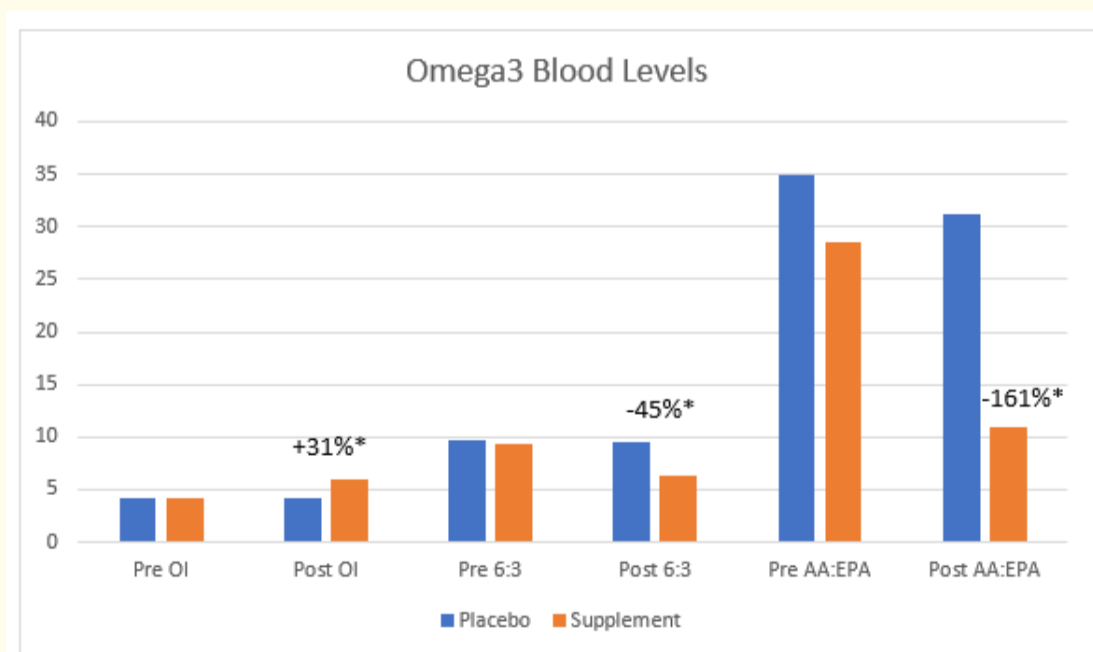
There was no significant change in global mood state (e.g. “overall well-being”), following the endurance run in the placebo group (Figure 2), while the supplement group demonstrated a 15% improvement (a lower number indicates a less negative psychological mood state).



**Figure 2:** Subjects in the supplement group had significantly better global mood state compared to placebo (lower score indicates higher well-being index). (\*Significantly different from post-supplementation placebo value,  $p < 0.05$ ).

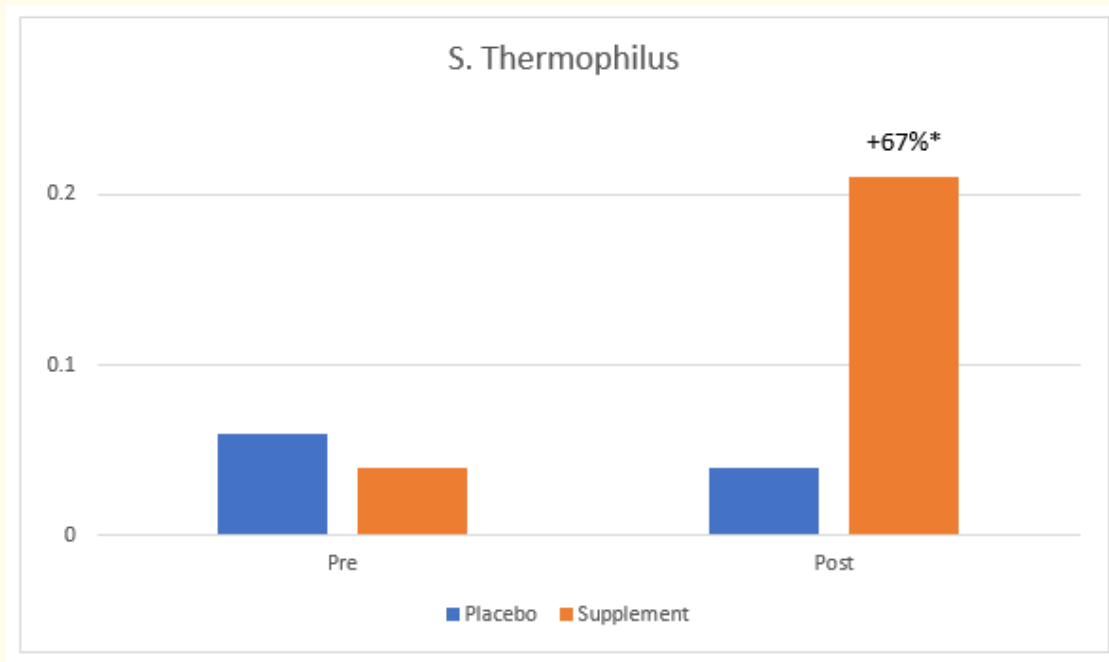
**Objective measures**

As expected, Omega3 blood values were significantly improved in the supplement group compared to placebo (Figure 3). The Omega-3 Index is the proportion of long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), of all fatty acids in red blood cell membranes - reflecting omega-3 status over the last 4 months. Omega-3 Index in the 8 - 12% range is generally considered to help to maintain heart, brain, eye and joint health. Omega-6:Omega-3 ratio is calculated by dividing the sum of seven omega-6 fatty acids by the sum of four omega-3 fatty acids in whole blood. Only one omega-6 fatty acid, arachidonic acid (AA), and one omega-3 fatty acid, eicosapentaenoic acid (EPA), make up the AA:EPA ratio. The desirable range for the Omega-6:Omega-3 ratio is 3:1 to 5:1 and the desirable range for the AA:EPA ratio is 2.5:1 - 11:1. Higher omega-3 blood levels are strongly related to improved health and longevity, particularly with health of the heart, brain and immune system.



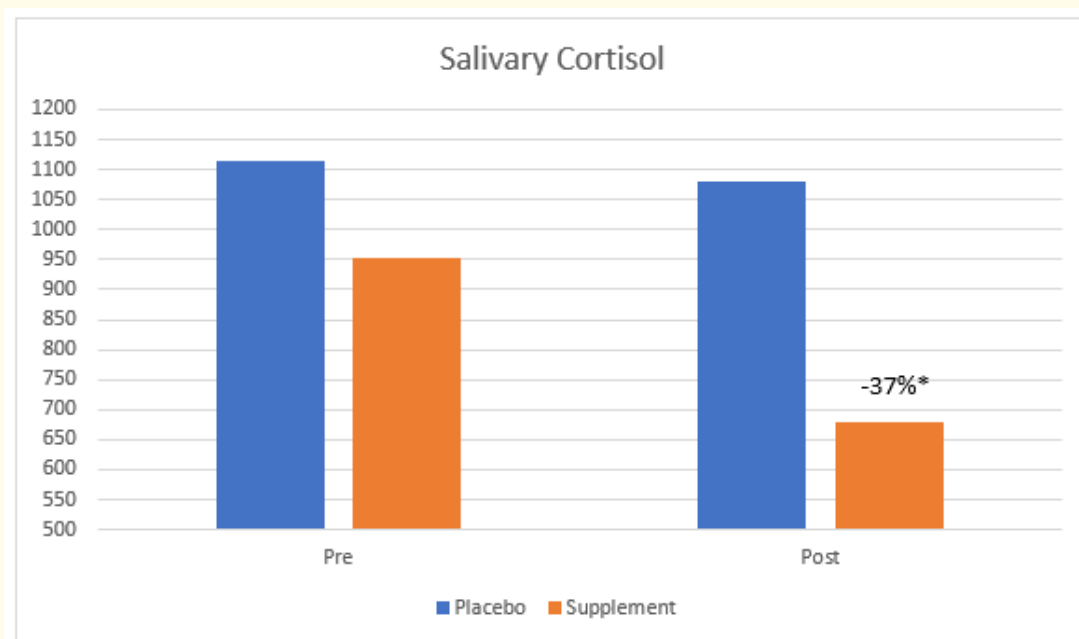
**Figure 3:** Subjects in the supplement group had significantly improved blood omega-3 profiles compared to placebo. (\*Significantly different from post-supplementation placebo value,  $p < 0.05$ ).  
 Abbreviations: OI = Omega3 Index; Reference Range 8 - 12% (higher is better).  
 6:3 = Omega6:3 Ratio; Reference Range 3-5:1 (lower is better).  
 AA:EPA = Arachidonic:Eicosapentaenoic Acid Ratio; Reference Range 2-11:1 (lower is better).

There was no significant difference in overall microbiome indices following the endurance run. However, following supplementation, *Streptococcus thermophilus* values were 67% higher in the supplement group, suggesting improved immune system regulation (Figure 4).



**Figure 4:** Subjects in the supplement group had significantly higher relative abundance of *Streptococcus thermophilus* bacteria compared to placebo. (\*Significantly different from post-supplementation placebo value,  $p < 0.05$ ).

Cortisol, the primary stress hormone related to both mood and immune function, was slightly but not significantly elevated in the placebo group following the endurance run, but was 37% lower in the supplement group post-supplementation compared to placebo (Figure 5).



**Figure 5:** Subjects in the supplement group had significantly lower salivary cortisol (ng/ml) compared to placebo. (\*Significantly different from post-supplementation placebo value,  $p < 0.05$ ).

## Discussion

These results demonstrate a significant and meaningful benefit of supplementation with a combination of black cumin seed oil plus fish oil for the immune system as both a “shield” (protection from upper-respiratory complaints) and as a “communication organ” (signaling well-being between body and mind and resulting in superior psychological mood state). This linkage between body and mind across the “Gut-Immune-Axis” [26] involves many aspects of a coordinated and interconnected communication system linking the gut microbiome (*S. thermophilus*) to the brain (psychological mood state) across the axis (immune and stress response pathways). When the entire system is balanced, as evidenced here in the supplement group, there is a noticeable benefit for physical health and mental wellness.

In order to “stress and suppress” the immune system of volunteers in our study, participants trained for and completed a strenuous endurance running event (half-marathon to marathon distance) to induce both physical and mental stress, as well as to create a “susceptibility window” whereby a higher risk for upper-respiratory tract complaints (URTCs) is more likely to be observed following intense endurance competition. Our hypothesis was that the group supplementing with the blend of black seed/fish oil as a natural immune modulator would demonstrate fewer URTC symptoms and lower indices of mental/physical stress.

Rather than being an “immune-booster” to stimulate immune system activity, both black cumin seed oil and fish oil may be considered as natural “immune-modulators” that can help to balance overall immune system activity. Such natural substances represent an emerging approach to immunotherapy that either elevates a suppressed immune system “up” to optimal - or calms an over-activated immune system “down” to optimal - in a paradigm that we refer to as “priming” the immune system. A properly primed immune system “pays attention” better to factors that it should fight (e.g. viruses, bacteria, cancer cells, etc.), while “ignoring” factors that should be considered non-harmful (e.g. pollen, mucus membranes, joint cartilage, etc).

The majority of both the microbiome and the immune system reside in the gut - forming a symbiotic relationship and ensuring that the human body is protected from harmful pathogens entering the body via the gut from ingested foods and liquids. Over time, our immune system shapes the diversity of our microbiome, and our gut influences the development and vigilance of our immune system. For example, the gut microbiome acts as a gatekeeper and a trainer and increasingly as a communication organ - educating and orchestrating immune function. In addition, the gut microbiome interacts with the brain in multi-directional ways that involve the immune system using neural, inflammatory, and hormonal signaling pathways [26]. These immune-mediated signals from gut to brain have been implicated in many aspects of mental health and well-being, including depression, anxiety, stress reactivity and overall psychological mood states [26].

In this study, we report the effect of supplementing with a combination of black seed oil plus fish oil for 4 weeks on the physical and psychological well-being of long-distance runners. The current study employed a series of subjective self-assessment questionnaires that addressed overall health status and URTCs. In addition to evaluation of subjects for physical health, a psychological assessment known as the Profile of Mood States (POMS) was conducted to assess mood state. We also collected objective markers of microbiome balance (*Streptococcus thermophilus*) and stress hormones (salivary cortisol), both of which are associated with immune system vigilance and psychological stress response, and which may represent a possible mechanism by which immune function and psychological mood state are related.

During the course of the 4-week treatment period (3 weeks before and 1 week after an intense endurance run), subjects in the supplement group reported fewer URTCs, better overall health and a more positive mood state compared to placebo. In addition, supplemented runners also showed higher levels of *Streptococcus thermophilus* (*S. thermophilus*) and lower stress hormone exposure (cortisol) - both of which being associated with immune vigilance and psychological mood state.

Runners and other athletes, whose athletic activities cause significant levels of both physical and mental stress, are more susceptible to URTI (infections) and URTC (complaints). Previous research has reported that athletes training for a marathon experience a deterioration

in global mood state [1,2,4-7,10,11,13,26], and a number of studies have reported that nutritional supplementation can modulate their health status [5,8,9,14-22].

Physical and psychological factors of subjects undergoing stressful situations are reported to increase URTI and URTC [12,13]. In all cases, the subjects supplemented with the black seed/fish oil combination experienced better physical health and improved psychological status (Global Mood State), than those in the placebo group. Supplemented subjects reported both fewer URTC symptoms and a better overall health status. The URTC symptoms reported by subjects are typical of cold and flu symptoms, and analogous to symptoms reported in other studies [5,12,13].

The POMS assessment for psychological health strongly supported and mirrored the physical health assessment. Illness and stress impact the immune system in both physical and psychological ways [13,26]. The POMS methodology has been used in more than 3,000 studies [25]; thus it has well-established validity and is sensitive to changes in psychological states pre/post-intervention. The survey instrument employs 65 adjective based scales that are scored by subjects without knowledge of how the scale scoring will be analyzed. The POMS survey instrument assesses the overall global mood state of subjects - analogous to a measurement of overall well-being and mental resilience.

Previous work has shown benefits of a range of dietary supplements may help reduce upper-respiratory symptoms in athletes [5,8,9,14-22], i.e. beta-glucans reduced URTCs and improved psychological mood state; zinc treatment reduced duration and severity of cold symptoms; probiotics (*Lactobacillus fermentum*) reduced the severity and duration of URTI in athletes; and vitamin C supplementation reduced the duration and severity of URTI when taken 21 days before a 90 km ultramarathon.

### Conclusion

In this study, the combination of black cumin seed oil plus fish oil significantly decreased upper-respiratory tract complaints and improved psychological mood state following intense endurance training and competition. These findings support a strong link for physical and mental benefits of the oil blend following the stress of endurance exercise. Additionally, subjects in the Supplement group had lower cortisol and superior microbiome parameters, suggesting that immune vigilance and mental well-being is linked through the microbiome and stress response pathways. These results add to the growing scientific literature and natural armamentarium for immune-modulation to both reduce URTI/URTC symptoms and improve psychological mood state in “stressed” individuals (endurance athletes in this study).

### Conflicts of Interest and Funding Statement

This study was funded by TriNutra, which manufactures and sells ThymoQuin black cumin seed oil and GC Reiber, which manufactures and sells fish oil supplements. The study was designed and conducted by 3Waves Wellness, which was compensated to carry out the trial. ST is an employee of Amare Global, which sells dietary supplements that include black cumin seed oil and fish oil. ST and JT are owners of 3Waves Wellness, an independent research organization.

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