ORIGINAL RESEARCH ARTICLE

The effects of an L-methionine combination supplement on symptoms of upper respiratory tract infections and performance in ultramarathon runners before, during and after ultra-endurance exercise

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Abstract

Objective. To evaluate whether supplementation with an L-methionine combination would reduce the incidence of upper respiratory tract symptoms (URTS) and improve performance in ultramarathon runners.

Design. A double-blind placebo-controlled study.

Setting. Twenty-one ultramarathon runners (17 males, 4 females) preparing for participation in an 87.3 km ultramarathon.

Interventions. L-methionine combination supplement (L-methionine, vitamin B_6, vitamin B_{12}, folic acid and magnesium) or placebo containing potato starch.

Main outcome measures. Incidence of URTS was recorded during the runner's preparation for an ultramarathon race (75 days) and recovery from the same (75 days). CD4+, CD8+ cell counts and ratios were measured pre race, immediately post race and 75 days post race. VO_{2max} and endurance fitness (percentage VO_{2max} at 4 $mmol^{-1}$ lactate concentration) were measured during the preparation period for the race.

Results. During the preparation period the incidence of URTS was 36% in the supplement group and 80% in the placebo group (p = 0.08). The incidence of URTS during the 3 weeks post race was 27% in the supplement group and 40% in the placebo group (p = 0.65). The CD4+/CD8+ cell ratios were not significantly different between groups. Endurance fitness prior to the race and race times were not significantly different.

Conclusions. Although the findings of the current study show that an L-methionine combination supplement did not reduce the incidence of URTS or improve performance in ultramarathon runners, benefits may be found with a more detailed investigation using larger sample sizes and immunosuppressed athletes.

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Introduction

A high incidence of upper respiratory tract infections (URTIs) has been reported in athletes.[7,19,21,22] These infections occur during periods of intense training and after major competitions such as ultramarathon events.[28] To compete successfully in these ultramarathon events athletes need to train at least daily, and often twice per day, and therefore it is possible that chronic suppression of immune function may result from the cumulative acute post-exercise suppression of each successive exercise bout.[14] The increase in infection risk after participation in intense exercise provides a model to study the efficacy of agents that may enhance resistance to such infections.[19,22]

A number of studies have investigated the impact of nutritional supplements on immune status and URTIs, with conflicting results being reported.[1,18,22] However, none have investigated the role of an L-methionine combination supplement (L-methionine, vitamin B_6, vitamin B_{12}, folic acid and

magnesium) on symptoms of URTIs in athletes. Results from a study investigating the effects of an L-methionine combination supplement in HIV+ patients propose that it slows the rate of decline in CD4+ cell counts and improves the viral load levels after 3 months.[32] The 5 active substances (L-methionine, vitamin B_6, vitamin B_{12}, folic acid and magnesium) in the supplement participate in the maintenance of glutathione status and have been shown to elevate intracellular glutathione.[16,31,33]

Magnesium is an essential co-factor for the enzyme methionine adenosyl transferase, which forms S-adenosylmethionine (SAM) from L-methionine.[16] SAM can provide a methyl group to a variety of substances and S-adenosylhomocysteine (SAH) is formed as a product.[16] SAH can then form L-homocysteine and adenosine.[16] Folate and vitamin B_{12} are essential co-factors for the remethylation of L-homocysteine to L-methionine. SAH can be converted by 3 enzymatic steps (which involve vitamin B_6) to L-cysteine and subsequently to glutathione.[16] It is glutathione (a major endogenous antioxidant) that has been found to have antiviral activity and to be important as a mediator of normal immune responsiveness.[9,11] Strenuous exercise has been shown to deplete tissue glutathione content[12,13] and increase the risk of URTIs.[19]

In addition to studying the effects of antioxidant supplements on immune status and URTIs, a number of studies have also investigated their role in performance.[23,25,30,34] Although several studies have indicated that supplementation with vitamins E and C decreases exercise-induced oxidative stress, there is little evidence that antioxidant supplementation can improve human performance.[24] However, one well-designed study has reported an improvement in muscular endurance following supplementation with N-acetylcysteine (a glutathione precursor).[25]

Therefore, the purpose of this study was to use a double-blinded, placebo-controlled design to determine firstly whether supplementation with an L-methionine combination supplement would decrease the incidence of symptoms of URTIs in runners during their preparation and recovery from an ultramarathon race, and secondly whether this supplement would impact on their endurance fitness prior to the ultramarathon and ultimately how it would influence their performance in the ultramarathon.

Methods

Subjects

Prior to commencement of this study, ethics approval was obtained from the Committee for Research on Human Subjects of the University of the Witwatersrand.

Thirty healthy well-trained, ultramarathon runners (20 males and 10 females) preparing for participation in an 87.3 km ultramarathon race volunteered to participate in this study after providing informed consent. Runners reported having run on average 5 ± 3 (mean ± SD) ultramarathons, with the best marathon performances averaging 3.17 ± 0.22 (mean ± SD) hours.

Subjects were paired according to VO_{2max} levels, proposed training programmes and gender. Each member of a pair was randomly assigned to either the supplementation (experimental) or placebo group.

During the training period prior to the race 5 subjects withdrew from the study due to orthopaedic injuries while 4 subjects failed to comply with testing procedures and were asked to discontinue with the study. The dropout rate in the current study (N = 9) was high but comparable with that reported in other studies (N = 8).[22,23] The sample was finally fixed at 21 subjects, which included 4 females and 17 males (N = 11 supplement and N = 10 placebo). Of the final 21 subjects, 6 subjects in each group remained paired according to the criteria stated above.

Performance measurements

The subjects were required to report to the testing laboratory situated at an altitude of 1 330 m with an average barometric pressure of 656 mmHg and a room temperature regulated at 20 - 22ºC on day 1 (75 days before the race). All subjects were tested within the same week. The subjects were familiarised with the testing equipment and procedures prior to the commencement of testing. Subjects were asked not to train for 12 hours prior to all the testing, not to train hard (≤ 70% of maximal heart rate) on the day before testing and not to consume any food or caffeinated drinks 3 hours prior to testing.

Each subject was asked to run on a motorised treadmill (Quinton Q65 series 90, Quinton Instrument Co., Bothell, WA, USA). Maximal aerobic capacity (VO_{2max}) was measured using a continuous model adapted from the Astrand protocol.[15] The criteria for VO_{2max} included subjects obtaining 2 of the following 3 criteria: a plateau in VO_2 despite an increase in workload, a respiratory exchange ratio in excess of 1.10, and blood lactic acid levels > 8 $mmol.^{-1}$.[6] Oxygen consumption and ventilation were determined using the MedGraphics CardiO_2 combined VO_2/ECG exercise system (Medical Graphics Corporation, St. Paul, MN, USA).

The lactate concentration for capillary blood was measured using the Accusport Analyzer (Boehringer Mannheim, BmbH, Mannheim, Germany) and the BM-Lactate test strips. Blood samples were collected during the last 30 s of each workload during the VO_{2max} test. Capillary blood was obtained by pricking a fingertip with a lancet. On average 5 blood samples were obtained during each VO_{2max} test. Lactate concentrations at each workload were plotted and an exponential curve was fitted using Table Curve 2D (Jandel Scientific Software, San Rafael, CA, USA). The percentage VO_{2max} at 4 $mmol.^{-1}$ lactate was calculated and used as a measure of endurance fitness.[8,10] The above tests were repeated again 14 days before the race. All tests were again performed within 1 week and at the same time of the day as the first testing session.

In addition to endurance fitness, race time (as obtained from official race records) was also used as a measure of performance.

Supplementation

Supplementation started on day 2 and ended on day 150 of the study. The supplement (Bio Boost) and placebo were supplied by Biomox Pharmaceuticals in capsule form and administered orally. The active ingredients administered in

the supplement were: L-methionine 405 mg, folic acid 0.36 mg, vitamin B_6 1.575 mg, vitamin B_{12} 0.01125 mg, magnesium 135 mg. The placebo contained potato starch (500 mg per day) and no active ingredients. From day 1 to day 7 (first week of the study) and day 76 to day 83 (first week after the race), subjects ingested 3 capsules twice a day (1 215 mg of L-methionine, 1.08 mg folic acid, 4.725 mg vitamin B_6, 0.03375 mg vitamin B_{12} and 405 mg magnesium) on an empty stomach. After these 2 periods (i.e. days 8 - 75 and days 84 - 151) subjects were instructed to ingest 2 capsules twice a day (810 mg L-methionine, 0.72 mg folic acid, 3.15 mg vitamin B_6, 0.0225 mg vitamin B_{12} and 270 mg magnesium) on an empty stomach. This schedule of ingestion was prescribed by the supplier and manufacturer of the product based on trials done on individual patients by the original formulators (unpublished data) and was the same for the 2 groups.

Incidence of upper respiratory tract symptoms (URTS)

The incidence of symptoms of URTIs was recorded daily by the subjects in a logbook provided which was based on the questionnaire used in the studies investigating the effect of glutamine or placebo ingestion on the incidence of infections in runners.[2] The adapted questionnaire was not validated prior to use in this study. Subjects were asked to record all symptoms reported under the headings of cold, cough, sore throat, running nose, sneezing and influenza and for how long the symptoms were present.[2] A subject was considered to have an illness when he or she reported 3 symptoms for a cold (cough, sore throat, running nose, sneezing) or influenza (fever, aches and pains in joints or muscles, cough, sore throat) such that they did not train or such that they consulted a doctor for treatment. Other illnesses, e.g. diarrhoea and vomiting were not included. The incidence of the symptoms and the duration of the symptoms (number of days the symptoms were present) was calculated. Recordings were controlled monthly with each visit to the laboratory to collect more supplements and telephonically when booking each appointment. The diagnosis was not verified by clinical examination although patients did indicate if they had seen a medical doctor for treatment.

Blood sampling

CD4+ and CD8+ measurements were taken on day 75 (18 hours pre-race), day 76 (10 min post race) and on day 150. With each sampling session blood was drawn from a forearm vein into evacuated collection tubes. The blood for the CD4+ and CD8+ measurements was collected in a 4.5 ml vacuette (K3E EDTA K3, Greiner Labortechnik). Following processing, specimens were packed in a refrigerated container and transported to a central laboratory where they were analysed within 18 hours of collection. The proportions of T-cells (CD4+ and CD8+) were measured using flow cytometry (FACSCount, Becton Dickinson, San Jose, USA) which includes the use of fluorescent labelled monoclonal antibodies to cell surface antigens as previously described.[26] All concentrations were corrected for changes in plasma volume post race.[3]

Training

All training information (running distance, time and intensity) and injuries were recorded weekly in a standardised training logbook given to the subjects. The training logbooks were adapted from those used in a study investigating the effects of lactate-correlated training on running performance but were not validated prior to use in this study.[27]

Nutrition

The subject's compliance with capsule use was recorded daily in a logbook given to the subjects. In addition, every 4 weeks subjects were asked to indicate how many capsules were left before they received a new bottle of capsules. Subjects were asked not to take any other vitamin and mineral supplements for the duration of the study. Dietary habits were evaluated from a 24-h dietary recall. As a method of reporting food intake the 24-h dietary recall questionnaire has been validated against weighted 7-day food records.[4] The dietary recall was completed daily by each subject to ensure that subjects did not change their diets over the duration of the study. Subjects were taught how to keep accurate food records prior to commencement of the study. Nutrient content of each athlete's reported daily dietary intake was assessed (macro and micro nutrients) using a computerised dietary analysis system (Food Fundi Analyzer 2, Professional Penta Medical Systems, Johannesburg, South Africa).

Statistical analysis

Data are expressed as means ± standard deviation (SD). The analysis of changes in all blood measurements (CD4+, CD8+ cell counts and ratio) was done using an analysis of variance (ANOVA) procedure for repeated measures to estimate main effects (group or time) and an interaction effect (group x time) followed by a Tukey-Kramer multiple comparisons post-hoc test. For all other dependent variables the unpaired *t*-test was used to test for the significance of the differences between the groups. A Fisher's exact probability test was used to analyse a 2 x 2 contingency table for being sick and not being sick (incidence of illness). The level of significance used was $p \leq 0.05$. A *post hoc* power analysis showed the power for the following statistical tests: repeated measures ANOVA (time effect power = 0.990, time x group effect power = 0.153); unpaired *t*-tests (%VO_{2max} @ 4mmol.$^{-1}$ lactate power = 0.7968, duration of symptoms power = 0.4264) and Fisher's exact test (incidence of illness power = 0.3196).

Results

Physical characteristics

The physical and performance characteristics of the subjects are given in Table I. There was no significant group differences on any of the parameters measured. It should be noted that based on the race finishing times and VO_{2max} values the runners would be classified as non-elite, experienced and well-trained runners.[20]

TABLE I. Physical and performance characteristics of the subjects*

	Supplement group (*N* = 11)	Placebo group (*N* = 10)	*P*-value
Age (years)	36 ± 3	35 ± 8	0.74
Height (m)	1.73 ± 0.09	1.75 ± 0.05	0.67
Mass (kg)	69.5 ± 10.7	68.9 ± 8.4	0.89
VO_{2max} ($ml.kg^{-1}.min^{-1}$)	58 ± 8	57 ± 7	0.78
Best standard marathon time (hours)	3.18 ± 0.25	3.16 ± 0.20	0.84
Best Comrades marathon (90 km) time (hours)	8.37 ± 1.09	8.27 ± 1.07	0.83

*Values are means ± SD.

Table III. Aerobic capacity variables monitored during preparation for the race

Parameters	April	June (12-14 days prior to the race)
VO_{2max} ($ml.kg^{-1}.min^{-1}$)		
Supplement	58 ± 8	59 ± 7
Placebo	57 ± 7	58 ± 7
Percentage VO_{2max} at 4 $mmol.^{-1}$ lactate concentration		
Supplement	86 ± 4	87 ± 4*
Placebo	84 ± 5	83 ± 5

Values are means ±SD; *N* = 11 supplement and *N* = 10 placebo subjects.
Difference between absolute values: April *P* > 0.05;*June *P* < 0.05
Difference between delta values (June - April); *P* = 0.08 for percentage VO_{2max} at 4 $mmol.^{-1}$ lactate concentration; VO_{2max} *P* = 0.84.

Incidence of upper respiratory tract symptoms (URTS)

The incidence of symptoms of URTIs in both groups during the 75 days of preparation is shown in Table II. During the preparation period the percentage of symptoms of URTIs (p = 0.08) and the duration of symptoms per illness incident (p = 0.13) was not significantly different. The incidence of URTS (p = 0.65) and duration of symptoms (p = 0.55) during the 3 weeks following the race was not significantly different. During the entire 75 days of recovery from the race the incidence of URTS (p = 1.00) and duration of symptoms was also not significantly different (p = 0.66).

Training programmes

There was no significant difference in the total training distance completed in preparation for the race, with the supplement group completing 896 ± 235 km and the placebo group 892 ± 192 km.

Physiological responses

Aerobic capacity variables monitored during preparation for the race are shown in Table III. The supplement group had a significantly greater percentage VO_{2max} at 4 $mmol.^{-1}$ lactate concentration (endurance fitness) than the placebo group 12 - 14 days prior to the start of the race. However the mean change in endurance fitness over the 75-day training period was not significantly different. No significant differences in race times were found, with the supplement group running 8.45 ± 1.54 hours and the placebo group 8.51 ± 1.08 hours.

Prior to the start of the race (18 hours) no significant differences were noted in the immune cell subsets (Figs 1 and 2).

CD4+ cells decreased significantly in both groups immediately post race (Fig. 1) while the CD8+ cells only decreased significantly in the supplement group immediately post race (Fig. 2). The CD4+/CD8+ ratio did not change significantly immediately post race in both groups or 75 days post race (Fig. 3). No significant differences were noted between the groups for the CD4+, CD8+ cell counts and ratios.

Nutrition

The subjects were all Caucasian, from the same socio-economic background and no significant differences (p > 0.05) between groups were observed for nutrient analysis of food records across all time periods. The average daily energy intake for all was 13 600 ± 4 009 $kJ.day^{-1}$, with carbohydrate, fat and protein respectively comprising 58 ± 11.5%, 24.2 ± 6.3% and 15.4 ± 3.5% of the daily caloric intake.

Discussion

The essential finding of this study was that 150 days of supplementation with an L-methionine combination had no significant effect on the incidence of URTS and race

TABLE II. Percentage of symptoms of URTIs and duration of symptoms during the study

	Subject/ group	Subjects with symptoms/total group	% symptoms of URTIs	P-value % symptoms	Mean duration of symptoms (days)	P-value mean duration of symptoms
Preparation (0 - 75 day)	Supplement	4/11	36		3 ± 1	
	Placebo	8/10	80	0.08	5 ± 3	0.13
3 weeks post-race (76 - 97 day)	Supplement	3/11	27		4 ± 2	
				0.65		0.55
	Placebo	4/10	40		6 ± 4	
During recovery (76 -150 day)	Supplement	6/11	55		6 ± 4	
				1.00		0.66
	Placebo	6/10	60		5 ± 3	

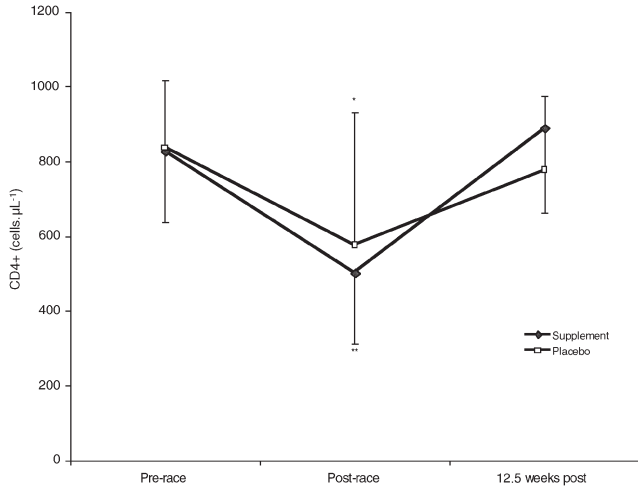

Fig. 1. CD4+ cell measurements in both groups pre-race, immediately post-race and 12.5 weeks post-race (** p < 0.001 for the supplement group and * p < 0.001 for the placebo group immediately post-race when compared with pre-race values). (Values are means ± SD; N = 11 supplement and N = 10 placebo subjects).

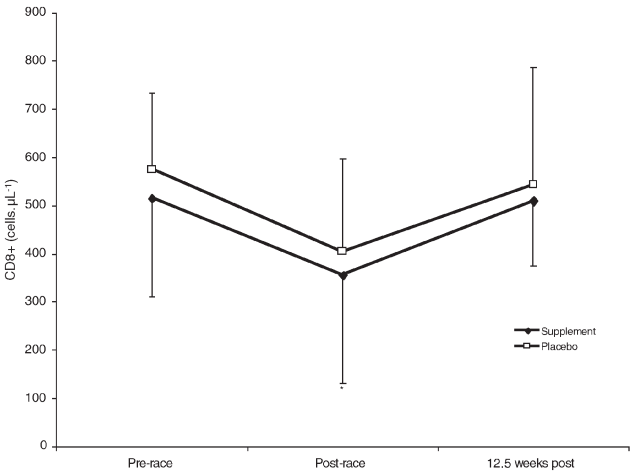

Fig 2. CD8+ cell measurements in both groups pre-race, immediately post-race and 12.5 weeks post-race (* p < 0.01 for the supplement group immediately post-race when compared with pre-race values). (Values are means ± SD; N = 11 supplement and N = 10 placebo subjects).

performance in ultra-endurance runners.

There is evidence for the role of nutrient supplements in modulating the incidence of URTIs post race. Peters *et al*.[22] examined the effect of supplementation with vitamin C (an antioxidant) on the URTIs of ultramarathon runners competing in the same race in 1990 and showed that 21 days of vitamin C supplementation reduced the post-race incidence of URTIs. Glutamine (an amino acid) supplementation immediately post race and 2 hours post race has also been shown to reduce the incidence of infections within 7 days post race in runners competing in marathon and ultramarathon events.[2]

The results of the current study show that supplementation with an L-methionine combination did not influence the

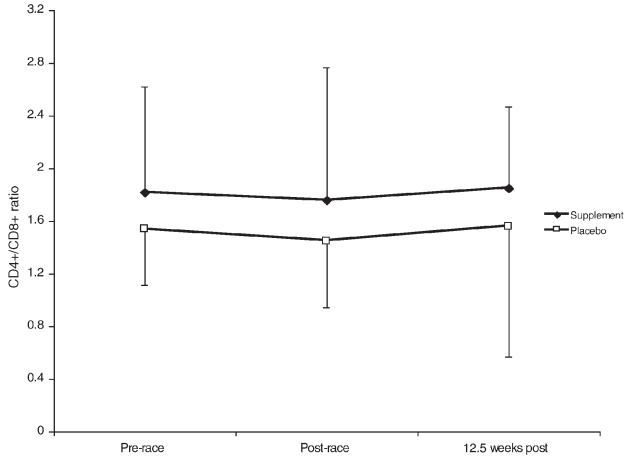

Fig 3. CD4+/CD8+ ratio measurements in both groups pre-race, immediately post-race and 12.5 weeks post-race. (Values are means ± SD; N = 11 supplement and N = 10 placebo subjects).

incidence of URTS during the training for or recovery from an ultramarathon race in runners nor did it reduce the duration of symptoms. One main difference between the above two studies and this study is the use of larger sample sizes (27 - 88 subjects). Owing to the long duration of the study 9 subjects withdrew, which is similar to what has been reported in other studies.[22,23] The small sample size used in the current study could influence the acceptance or rejection of the null hypothesis.

In addition to the results on URTS, we did not find any significant differences in the CD4+ and CD8+ cell counts or CD4+/CD8+ cell ratios post race. This immune marker was used as an L-methionine combination supplement (supplement with the same combination as used in this study but at a higher concentration) and N-acetylcysteine (NAC) treatment, both of which are glutathione precursors and have been shown to slow the decrease of CD4+ cell counts in HIV+ patients.[12,32] The stress of prolonged exhaustive exercise has been shown to lower the CD4+ cell counts and CD4+ to CD8+ cell ratio in athletes.[1] This marker has also been used in other studies which have examined the effect of supplementation on the immune system post marathon and it has been suggested that a ratio of CD4+/CD8+ cells below 1.5 is below normal and may be a cause of and an indicator of immunosuppression in athletes.[17,29]

There are, however, several factors which might account for the negative findings regarding the immune responses measured in this study. Firstly no CD4+/CD8+ cell measurements were taken during the training period in which the largest difference in the incidence of URTS was noted between the supplement and placebo groups. Secondly, enumeration of circulating cells does not provide as conclusive information on immune function or activation of cells as does examining direct measurements of cell function.[35] Thirdly, the reported symptoms of URTIs by the subjects were not verified by clinical examination which would have confirmed the presence of an infection. Fourthly, including a non-exercising control group would have strengthened the findings of the current study as the incidence of symptoms in the general population could have been compared with that

found in the exercising group. Furthermore it has been suggested that the CD4+ T-cell system may be negatively affected not only by suboptimal but also by supraoptimal glutathione levels.[12] Therefore further investigation is needed to not only analyse the activity of the immune cells in response to L-methionine combination supplementation but also in relation to glutathione levels.

In addition to the role of antioxidant supplements in immune function, previous studies have also examined their effects on performance due to growing evidence indicating that radicals and other reactive oxygen species may contribute to muscular fatigue.[24] Conflicting results have been presented from these studies which may be due to agent administration, dosage and the pattern of muscular activity used to induce fatigue.

Weight *et al*.[34] found that 3 months of vitamin and mineral supplementation had no effect on oxygen consumption, blood lactate turnpoint, peak treadmill running speed and 15 km time trial performance in 30 well-trained runners. Time to exhaustion at 70% VO_{2max} was not reduced in 11 highly trained male triathlon athletes after 4 weeks of supplementation with vitamin E, coenzyme Q10, cytochrome C and inosine.[30] Reid *et al*.,[25] however found an improvement in muscular endurance (during low frequency stimulation) following treatment with 150 mg N-acetylcysteine (a glutathione precursor). N-acetylcysteine has been shown to increase glutathione levels in plasma and bronchoalveolar lavage fluid in humans; however its use is limited due to a number of side-effects.[25] In the present study no change in VO_{2max} and endurance fitness (percentage VO_{2max} at 4 $mmol.^{-1}$ lactate) was found after 75 days of supplementation. The finding that performance in the race (87.3 km) was unaltered by supplementation tends to confirm the laboratory findings. These findings are therefore in keeping with the majority of studies showing that long-term supplementation with antioxidant nutrients does not improve human performance.[5,24,34]

Conclusions

In summary, although the findings of the current study do not support the use of an L-methionine combination supplement in reducing the incidence of URTS in ultramarathon runners, the positive results found with HIV+ patients does suggest that this supplement has a role to play in immune function. Future studies will need to be conducted with larger sample sizes, will need to include measures of immune function and glutathione levels, and will need to be directed at athletes who are shown to be immunosuppressed such as overtrained athletes or patients who present with specific disease states in which glutathione or its precursors are depleted.

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