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Quercetin reduces susceptibility to influenza infection following stressful exercise

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Davis JM, Murphy EA, McClellan JL, Carmichael MD, Gangemi JD. Quercetin reduces susceptibility to influenza infection following stressful exercise. *Am J Physiol Regul Integr Comp Physiol* 295: R505–R509, 2008. First published June 25, 2008; doi:10.1152/ajpregu.90319.2008.—Exercise stress is associated with increased risk for upper respiratory tract infection. We have shown that exercise stress can increase susceptibility to infection. Quercetin, a flavonoid present in a wide variety of fruits and vegetables, has been reported to inhibit infectivity and replication of a broad spectrum of viruses and may offset the increase in susceptibility to infection associated with stressful exercise. This study examined the effects of quercetin feedings on susceptibility to the influenza virus A/Puerto Rico/8/34 (H1N1) following stressful exercise. Mice were randomly assigned to one of four treatment groups: exercise-placebo, exercise-quercetin, control-placebo, or control-quercetin. Exercise consisted of a run to fatigue (~140 min) on a treadmill for 3 consecutive days. Quercetin (12.5 mg/kg) was administered via gavage for 7 days before viral challenge. At 30 min after the last bout of exercise or rest, mice (n = 23–30) were intranasally inoculated with a standardized dose of influenza virus (0.04 hemagglutinating units). Mice were monitored daily for morbidity (time to sickness), symptom severity, and mortality (time to death) for 21 days. Exercise stress was associated with an increased susceptibility to infection [morbidity, mortality, and symptom severity on *days 5–7* ($P < 0.05$)]; quercetin offset the increase in susceptibility to infection [morbidity, mortality, and symptom severity on *days 5–7* ($P < 0.05$)] that was associated with stressful exercise. These data suggest that short-term quercetin feedings may prove to be an effective strategy to lessen the impact of stressful exercise on susceptibility to respiratory infection.

nutrition; mice; infection; stress; virus

SEVERAL STUDIES HAVE REPORTED that exercise can alter many immune system components and, therefore, modulate susceptibility to infection. It has been hypothesized that exercise stress can increase the risk for upper respiratory tract infection (URTI); data from controlled experimental animal studies generally support this hypothesis (5, 6, 11, 13, 19, 22), although there is some controversy regarding the supporting evidence in human subjects (4). There is very little evidence of the effects of exercise stress on susceptibility to the principal etiological agents of human respiratory infections, including influenza viruses. Our laboratory has used a herpes simplex virus (HSV-1) and an influenza [A/Puerto Rico/8/34 (H1N1)] mouse model of respiratory infection to determine the effects of exercise stress on susceptibility to infection (5, 6, 11, 13). We have reported that exercise stress can increase susceptibility to HSV-1 and influenza infection [morbidity (time to sickness), symptom severity, and mortality (time to death)] (5, 6, 11, 13), which are associated with a decrease in macrophage antiviral resistance (11, 13). The effects associated with exercise stress are not unlike those that have resulted in other stress paradigms, such as psychological stress, which has been shown to alter infectivity of a variety of pathogens, including HSV-1 and influenza (2, 18, 34).

Various nutritional strategies, including β-glucan (13), carbohydrates (26, 28), glutamine (7), zinc (30), and antioxidants (29), have been investigated as possible countermeasures for immunosuppression during periods of stressful training and competition. Several of these strategies have been shown to alter many immune system components during periods of stressful exercise (13, 26, 28, 30). However, except for a beneficial effect of oat β-glucan on susceptibility to HSV-1 infection following exercise stress (13), there is little evidence to suggest that these or any other nutritional strategies produce clinically significant changes in susceptibility to infection (23).

Quercetin, a flavonoid present in a wide variety of fruits and vegetables, has been investigated for its antiviral activity. Cell culture studies have shown that quercetin can reduce infectivity of target cells and replication against a wide variety of respiratory viruses, including HSV-1 and HSV-2 (9, 14), adenoviruses (AdV-3, AdV-8, and AdV-11) (9), coronavirus (14), parainfluenza virus type 3, respiratory syncytial virus (20), rhinovirus (15), and severe acute respiratory syndrome (8). It has recently been reported that quercetin feedings can reduce URTI following 3 days of exhaustive cycling exercise in athletes (27). However, the effects of quercetin on susceptibility to infection following exercise stress in a controlled experimental viral challenge model have not been determined.

The purpose of the present study was to determine the effects of quercetin feedings on susceptibility to infection following stressful exercise in mice. Altered susceptibility to infection was measured as changes in morbidity (time to sickness), symptom severity, and mortality (time to death). This was done using a well-characterized murine model of exercise stress (5, 6, 11, 13) and respiratory infection (5, 6, 11–13, 24). The exercise protocol consisted of 3 consecutive days of prolonged treadmill running to fatigue designed to mimic a short period of severe exercise training. The virus used to induce respiratory infection, the influenza virus A/Puerto Rico/8/34 (H1N1), was administered after the 3 days of exercise stress. There is a lack of evidence of the effects of exercise

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stress or nutrition on influenza virus infection, one of the most common human respiratory viruses among military personnel and athletes. Furthermore, because there is no virus challenge pool for influenza virus infection studies in humans, animal models are the only tool to examine exercise and nutritional effects on susceptibility to influenza virus infection. Quercetin was used because of its documented widespread health benefits, which include antiviral activity, abundance in the diet, and reported lack of side effects when used as a dietary supplement or food additive (17). We hypothesized that quercetin would offset the exercise stress-induced increase in susceptibility (morbidity, symptom severity, and mortality) to influenza virus.

METHODS

Animals. Four-week-old male ICR mice (Harlan Sprague Dawley Labs) were acclimated to our facility for ≥3 days before any experimentation. Mice were purchased as pathogen-free stock, and periodic screening of sentinel mice yielded negative results for common murine viral or bacterial pathogens. Mice were housed, five per cage, and cared for in the animal facility at the University of South Carolina School of Medicine. Mice were maintained on a 12:12-h light-dark cycle in a low-stress environment (22°C, 50% humidity, low noise) and given food (Purina chow) and water ad libitum. All experiments were performed at the beginning of the active dark cycle. Animals were removed from the experiment if they refused to run on the treadmill, did not survive the inoculation, or expelled the inocula by sneezing. Typically, in our hands, this results in elimination of <10% of animals. The Institutional Animal Care and Use Committee of the University of South Carolina approved all experiments.

Quercetin treatment. Mice were randomly assigned to one for four treatment groups as follows: exercise-placebo (Ex-Plac, $n = 23$), exercise-quercetin (Ex-Q, $n = 27$), control-placebo (Con-Plac, $n = 30$), or control-quercetin (Con-Q, $n = 29$). Quercetin (12.5 mg/kg; catalog no. QU995, Quercegen Pharma, Newton, MA) was fed to the mice via gavage daily in 200 μl of Tang (Kraft Foods, Northfield, IL) for the 7 days before viral challenge. Quercetin was administered in Tang, which contains vitamins (especially B_3 and C) that have been suggested to increase the bioavailability of quercetin (personal communication, Quercegen Pharm). The placebo groups received Tang only during this time. The dose and timing of quercetin administration were based on evidence from our laboratory involving other effects of quercetin in rodents (unpublished data).

Treadmill acclimation and exercise protocol. The Institutional Animal Care and Use Committee of the University of South Carolina approved the protocol. The exercise groups were acclimated to the treadmill for 20 min/day for the 3 days before the experimental exercise bouts. The exercise protocol consisted of an exhaustive exercise bout of treadmill running (performed in the evening, at 6 PM) for 3 consecutive days. Mice in the exercise groups ran on the treadmill (2 per lane) at 36 m/min and 8% grade until they reached volitional fatigue. Fatigue was defined as the inability of the mouse to maintain the appropriate pace, despite continuous hand prodding for 1 min, at which time the mouse was removed from the treadmill. Electric shock was never used, inasmuch as mice readily respond to a gentle tap of the tail or hindquarters encouraging them to maintain pace with the treadmill. Mice rarely required this type of continual prodding until they approached the point of fatigue. The control groups remained in their cages in the treadmill room throughout the exercise bouts; they were exposed to similar handling and noise in an attempt to control for extraneous stresses that may be associated with treadmill running. The control groups were deprived of food and water during the exercise sessions.

Intranasal infection with influenza. On the day of the experiment, mice were exposed to control treatment or exercise. Immediately after exercise or control treatment, mice were given their final quercetin or placebo treatment and were returned to their cages; 30 min later they were lightly anesthetized with isoflurane and inoculated intranasally with 50 μl of a standardized dose (0.04 hemagglutinating units) of influenza virus [A/Puerto Rico/8/34 (H1N1)]. This dose yielded a ~50% mortality rate among control mice in preliminary dose-response experiments. After infection, the mice were returned to their respective cages and housed in an isolated BSL-2 (Biosafety level 2) facility. All animals were monitored daily for 21 days for signs of morbidity, symptom severity, and mortality by an investigator blinded to the treatments.

Measures of morbidity. Several typical symptoms of illness, including ruffled fur, redness around the eyes, nose, or mouth, altered respiration, hunched-back pose, and unresponsiveness, were included in the symptom severity scale (Table 1). Animals were scored daily on the basis of symptoms; each symptom was given a score of 1–3 depending on its severity. Symptoms that generally appear at the onset of sickness (ruffled fur and redness around the eyes) were weighted less than symptoms that appear as the illness became more severe (hunched-back pose, altered respiration, and unresponsiveness). In this model, symptoms such as ruffled fur and redness around the eyes will appear first (*days 4–5*) followed by altered respiration and a "hunched-back" pose (*days 5–6*); mice that are severely ill will be unresponsive (after *day 6*). Mice that displayed any of these sickness symptoms were considered morbid. Cumulative scores ranged of 0–9 were based on the varying degree of symptoms of sickness.

Statistical analysis. Statistical analyses were performed using a commercially available statistical package (version 2.03, SigmaStat, SPSS, Chicago, IL). Differences in morbidity (time to sickness) and mortality (time to death) between groups across the 21-day postinfection period were determined using the Lifetest Survival Analysis program in SigmaStat ($P < 0.05$), which reported mean and median time to sickness and death. Differences in symptom severity over the 21-day postinfection period were determined using a two-way analysis of variance (exercise × treatment) with Student-Newman-Keuls post hoc analysis ($P < 0.05$).

RESULTS

Morbidity. We examined the effects of quercetin on morbidity rates in mice after a controlled viral challenge with influenza virus and exercise stress. Morbidity is defined as *day 1* of onset of sickness symptoms. Group differences in morbidity were evident over the 21-day postinfection period (Fig. 1). Intranasal administration of influenza virus following 3 days of exercise stress resulted in an increase in morbidity (i.e., time to sickness) compared with resting controls ($P < 0.001$); mean time to sickness was 6.9 ± 0.7 (SE) days for Ex-Plac and 12.4 ± 1.4 days for Con-Plac. Median time to sickness (50% percentile) was 6.0 ± 0.19 days for Ex-Plac and 7.0 ± 0.6 days for Con-Plac. Ex-Plac experienced a 91% incidence in mor-

Table 1. *Symptom severity scale for influenza infection*

Score		
1	2	3
Red eyes Ruffled fur	Hunched back Altered breathing	Unresponsiveness

Animals were scored daily on the basis of symptoms. Cumulative scores ranged from 0 to 9 on the basis of the varying degree of symptoms of sickness. Symptoms that generally appear at the onset of sickness are weighted less (ruffled fur and redness around the eyes) than those that appear as the illness becomes more severe (hunched-back pose and unresponsiveness). Symptoms such as ruffled fur and red eyes will appear first (*days 4–5*) followed by altered breathing or a "hunched-back" pose (*days 5–6*); mice that are severely ill will be unresponsive (after *day 6*).

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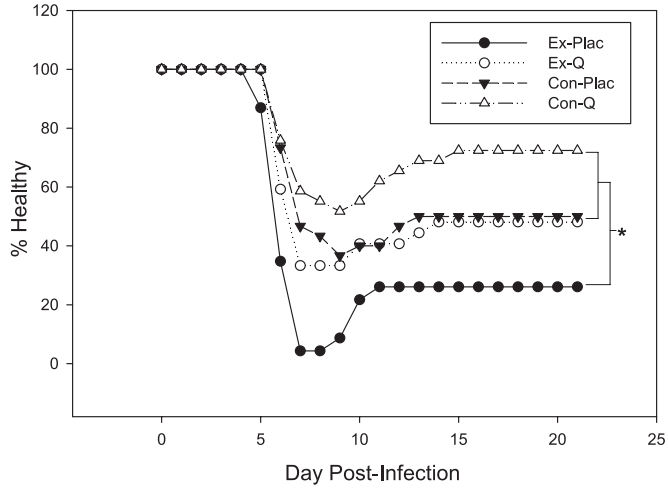

Fig. 1. Time course of morbidity across the 21-day postinfection period (n = 23–30/group). Ex-Plac, exercise-placebo; Ex-Q, exercise-quercetin; Con-Plac, control-placebo; Con-Q, control-quercetin. *$P < 0.01$, Ex-Plac vs. Ex-Q and Con-Plac vs. Con-Q.

bidity, while only 63% of Con-Plac got sick. Quercetin feedings for 7 days before infection significantly offset the increase in morbidity associated with stressful exercise ($P < 0.01$); mean time to sickness was 11.6 ± 1.5 days, and median time to sickness was 7.0 ± 0.35 days. Incidence of morbidity was 67% in Ex-Q. There was no difference between Ex-Q and Con-Plac and between Con-Plac and Con-Q.

Symptom severity. Sickness symptoms were also graded to better address possible differences in severity of sickness (Table 1). Morbidity data simply indicate the day on which the first symptom was observed. Symptom severity data for *days 1–7* (until and including death) after infection is presented in Fig. 2. Symptom scores are only reported to *day 7*, because as animals with very high symptom scores die, the mean scores for the living animals are artificially reduced, which could be inappropriately interpreted as recovery. In fact, very few animals recover once they become sick. Symptom severity was significantly different across the groups. The symptom severity score was significantly higher for Ex-Plac than Con-Plac on *days 5–7* ($P < 0.05$). However, quercetin feedings significantly offset the increased symptom score in Ex-Q on *days 5–7* ($P < 0.05$). There were no differences in Con-Plac vs. Ex-Q and Con-Q.

Mortality. Similar treatment effects were found for mortality (i.e., time to death) over the 21-day postinfection period (Fig. 3). Intranasal administration of influenza virus following 3 days of exercise stress resulted in a significant increase in mortality compared with resting controls ($P < 0.05$): mean time to death was 12.5 ± 1.3 days for Ex-Plac and 15.9 ± 1.2 days for Con-Plac. Median time to death (50% percentile) was 9.0 ± 0.33 days for Ex-Plac and 14.0 days for Con-Plac. Mortality was 74% in Ex-Plac compared with 50% in Con-Plac. Quercetin feedings for 7 days before infection significantly offset the increase in mortality associated with stressful exercise ($P < 0.01$): mean time to death was 16.5 ± 1.2 days and median time to death was 20.0 days. Mortality in Ex-Q was only 52%. There was no difference between Ex-Q and Con-Plac. There was, however, a trend toward a beneficial effect of quercetin in Con-Q ($P = 0.08$): mean time to death was 18.5 ± 1.1 days, and median time to death is not reported, inasmuch <50% of mice exhibited mortality. Mortality in Con-Q was only 28%.

DISCUSSION

Various nutritional strategies, including zinc and *Echinacea*, have been examined for their ability to enhance immune function and decrease susceptibility to infection. However, there is little evidence to suggest that these or any other nutritional strategies produce clinically significant changes in susceptibility to infection in healthy, nonimmunocompromised subjects (31, 32). Furthermore, evidence of a possible benefit of nutrition in offsetting the increased risk for respiratory infection that is associated with exercise stress is limited. Quercetin, a flavonoid, has been reported to have widespread health benefits (17), including antiviral activity (9, 14). Recent evidence has shown that quercetin feedings can reduce illness symptoms following 3 days of exhaustive cycling exercise (27); however, the results of this study were based on self-reported symtomatology, and there was no attempt to identify

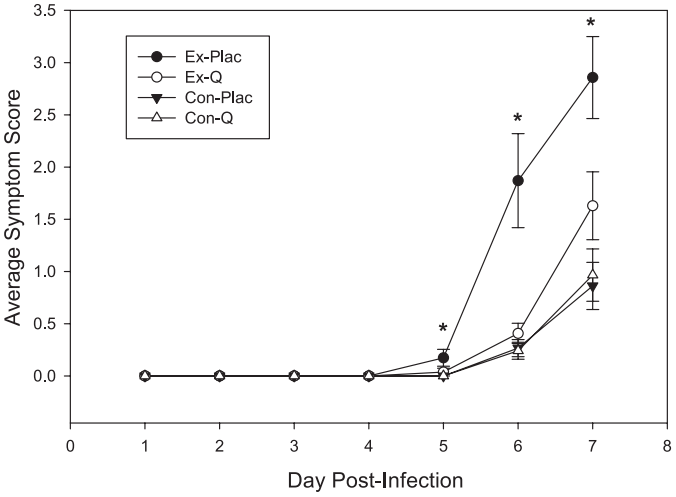

Fig. 2. Time course of symptom severity across the postinfection period (n = 23–30/group). Symptom severity was analyzed across groups on *days 1–7*. *$P < 0.05$, Ex-Plac vs. Ex-Q, Con-Plac, and Con-Q on *days 5–7*.

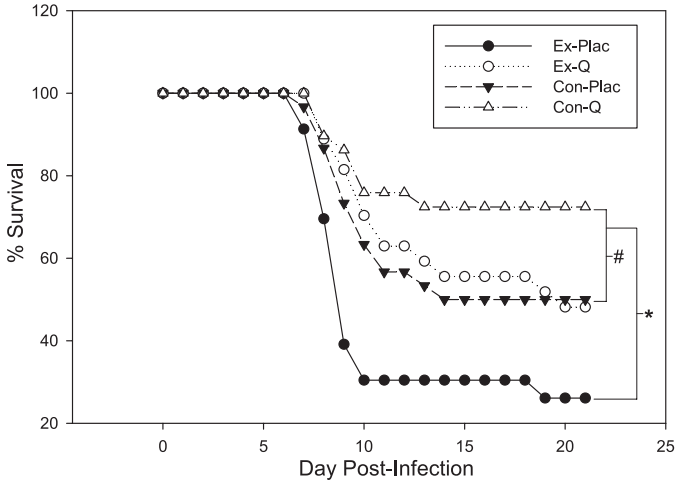

Fig. 3. Time course of mortality across 21-day postinfection period (n = 23–30/group). *$P < 0.05$, Ex-Plac vs. Ex-Q, Con-Plac, and Con-Q. #$P = 0.08$, Con-Plac vs. Con-Q.

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the pathogenic origin. The present study used an established model of exercise and respiratory infection to determine whether quercetin feedings could protect mice from influenza virus infection following 3 days of exercise stress. The data suggest that quercetin feedings can offset the exercise stress-induced increase in morbidity (time to sickness), symptom severity, and mortality (time to death) in mice after intranasal inoculation with a standardized dose of influenza virus [A/Puerto Rico/8/34 (H1N1)]. We also found a trend toward a decrease in susceptibility to infection in rested nonstressed mice after quercetin treatment.

Data from controlled animal studies have been important in determining the effects of exercise stress on susceptibility to respiratory infection. However, evidence of the effects of exercise stress on influenza virus, one of the most common respiratory viruses among soldiers and athletes, is still limited. The specific pathogenesis and symtomatology of influenza infection are well characterized in the literature (3). Several studies have used this model to determine the effects of exercise on susceptibility to infection and possible immune mechanisms (19, 21, 22). The results from this study indicate that 3 days of exhaustive exercise on a treadmill can increase susceptibility to influenza virus [A/Puerto Rico/8/34 (H1N1)] infection and are consistent with our previous findings and those of others. For example, exhaustive swimming resulted in a 33% increase in mortality following infection with influenza virus [A/Aichi/2/68 (H3N2)] (19), and prolonged treadmill running resulted in higher maximal morbidity scores following infection with influenza virus [A/Puerto Rico/8/34 (H1N1)] (22).

Quercetin has been reported to reduce infectivity of target cells and replication against a wide variety of respiratory viruses (8–10, 14, 15, 20). It has been shown to decrease multiplication and infectivity of several etiological agents of respiratory infection, including HSV-1 and HSV-2 (9, 14), AdV-3, AdV-8, and AdV-11 (9), coronavirus (14), parainfluenza virus type 3, respiratory syncytial virus (20), rhinovirus (15), and severe acute respiratory syndrome (8), in cell culture studies. Recent evidence in athletes shows that quercetin feedings can reduce self-reports of upper respiratory infection following 3 days of exhaustive exercise (27). Cyclists ingesting quercetin at 1,000 mg/day over a 3-wk period experienced a significantly lower incidence of infection during the 2-wk period following the intense exercise. It is important, however, to note that this was based on self-report of upper respiratory infection with no confirmation of viral illness. To our knowledge, these are the first data to show a benefit of quercetin on the exercise-induced increase in susceptibility to respiratory infection in a controlled experimental virus challenge model. This is especially important given that there is no influenza virus challenge pool for use in human subjects.

Our findings also indicate a potential benefit of quercetin in rested, nonstressed control mice, but this effect did not reach statistical significance. However, in studies such as this, it is not unusual to find a lack of effect in healthy nonstressed animals that becomes significant in immunosuppressed or stressed individuals. Furthermore, the present study was designed to examine a benefit of quercetin in exercise-stressed mice and the lethal dose was determined based on this purpose. It is certainly possible that the strong trend toward a benefit of quercetin on susceptibility to infection in rested, nonstressed mice may become significant if the viral dose was adjusted.

The precise mechanisms for the apparent beneficial effect of quercetin on susceptibility to infection following exercise stress are unknown. However, previous evidence suggests multiple possible mechanisms for the antiviral effects of quercetin. Cell culture studies have reported that quercetin can block viral replication at an early stage of multiplication for several respiratory viruses, including adenoviruses, coronaviruses, and rhinoviruses, and using several mechanisms, including inhibition of proteases by molecular docking, suppression of virulence enzymes such as DNA gyrase and cellular lipase, and binding of viral capsid proteins (8–10). It is also possible that quercetin's antiviral effects may be mediated through induction of interferon; quercetin induces the gene expression and production of helper T lymphocyte-1 (Th-1)-derived IFNγ, and it downregulates Th-2-derived IL-4 when cultured with human peripheral blood mononuclear cells (25). Studies have also reported that quercetin may enhance activity of a variety of immune system components. Data from in vitro and animal studies have shown that quercetin increases natural killer cell lytic activity, neutrophil chemotaxis, and mitogen-stimulated lymphocyte proliferation (1, 16, 25). Nieman et al. (27) examined the effects of quercetin feedings on immune system dysfunction following 3 days of prolonged cycling and reported no beneficial effects of quercetin on natural killer cell lytic activity, polymorphonuclear respiratory burst, or phytohemagglutinin-stimulated lymphocyte proliferation, despite the reduced incidence of upper respiratory tract infection symptoms following quercetin feedings. However, many immune system parameters that have been shown to play a role in susceptibility to respiratory infection following exercise stress could not be measured in that study, for example, lung macrophages, which have been shown to play a necessary role in elimination of virus following respiratory infection.

One of the findings of the present study suggests that short-term quercetin feedings before infection (i.e., primary prevention) can delay the onset of sickness following intranasal inoculation of the virus. Although it is easy to conclude, therefore, that quercetin protected the animals from infection, it is also possible that this was due to reduced inflammation, which would be expected to blunt the symptoms. Indeed, quercetin is known to have potent anti-inflammatory properties (17). Both of these outcomes would be considered positive responses to quercetin feeding, especially in this case, since overall morbidity and mortality were benefited by quercetin. However, in certain cases, a more rapid appearance and resolution of symptoms may be a more favorable outcome. It is important to note that a quercetin-induced reduction in inflammatory processes may not always result in a reduction in susceptibility to infection; it has been shown that inflammation is necessary for the efficient elimination of influenza virus infection (33). This is clearly an aspect that should be considered in future studies in which the dose and timing of quercetin administration in relation to virus challenge are investigated.

In conclusion, this is the first controlled experimental study to show a benefit of short-term quercetin feedings on susceptibility to respiratory infection following exercise stress. Quercetin feeding was an effective preventive strategy to offset the increase in susceptibility to infection (morbidity, symptom severity, and mortality) that was associated with stressful exercise. If our data can be clinically translated, they may lead to an important nutritional strategy to decrease the risk of

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infection, which can be a problem in athletes and military personnel, who are often exposed to combinations of severe physical, psychological, and environmental stress.

GRANTS

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