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The effect of vitamin C supplementation on incidence of upper respiratory tract infections in marathon runners

by [Himmelstein, Sharon Anne](#), Ph.D., **The University of New Mexico**, 1996, 163 pages; AAT 9709814

Abstract (Summary)

Several studies have found an increase in upper respiratory tract infections (URTIs) among distance runners (Nieman et al. 1990, Peters et al. 1983). Nieman et al. (1990) reported that marathon runners with higher training mileage had a two-fold increase in URTIs. Peters et al. (1993) found that vitamin C supplements reduced the incidence of URTIs among ultra-marathoners.

The hypothesis of the present study was that vitamin C supplementation does not affect the risk of URTIs among marathon runners. Marathon runners (n = 44) and sedentary subjects (n = 48) were randomly assigned either 1000 mg Vitamin C or a placebo daily for two months prior and one month following the 1994 Duke City Marathon.

Baseline plasma vitamin C concentrations were higher among the runners compared to sedentary subjects (\$1.38 \pm 0.05 and \$1.48 \pm 0.06 mg/dL for vitamin C and placebo-treated runners and \$1.08 \pm 0.08 and \$0.93 \pm 0.09 mg/dL for vitamin C and placebo-treated sedentary subjects). Vitamin C concentrations increased with supplementation, however the increase was more marked among the sedentary subjects (\$1.43 \pm 0.04 mg/dL for runners and \$1.28 \pm 0.05 mg/dL for sedentary subjects following two months of supplementation).

Although no treatment differences were found in URTI incidence for the 3 month period, vitamin C-treated runners tended to have fewer colds during the two week period following the marathon compared to placebo-treated runners (10.0% versus 28.6%).

Using multiple logistic regression, the following factors were related to risk of URTIs: (1) faster training pace, (2) greater number of marathons run, (3) running a shorter distance on the longest run of the week, and (4) female gender. Running mileage and stress levels were not related to risk of URTIs.

Vitamin C supplementation had no apparent effect on lymphocyte proliferation.

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**THE EFFECT OF VITAMIN C SUPPLEMENTATION
ON INCIDENCE OF UPPER RESPIRATORY TRACT INFECTIONS
IN MARATHON RUNNERS**

by

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DISSERTATION

Submitted in Partial Fulfillment of the
Requirements for the Degree of

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The University of New Mexico
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@ 1996, Sharon Anne Himmelstein

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Using multiple logistic regression, the following factors were related to risk of URTIs: (1) faster training pace, (2) greater number of marathons run, (3) running a shorter distance on the longest run of the week, and (4) female gender. Running mileage and stress levels were not related to risk of URTIs.

Baseline plasma vitamin C concentrations were higher among the runners compared to sedentary subjects ($1.38 \pm .05$ and $1.48 \pm .06$ mg/dL for vitamin C and placebo-treated runners and $1.08 \pm .08$ and $0.93 \pm .09$ mg/dL for vitamin C and placebo-treated sedentary subjects). Vitamin C concentrations increased with supplementation, however the increase was more marked among the sedentary subjects ($1.43 \pm .04$ mg/dL for runners and $1.28 \pm .05$ mg/dL for sedentary subjects following two months of supplementation).

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Chapter One

Introduction

There is strong evidence that regular exercise is beneficial for promoting health. Studies have shown that even low levels of activity can protect against hypertension, coronary heart disease, colon cancer, osteoporosis, and stroke. In addition, exercise tends to promote weight control and psychological well-being.

Indeed, not only is exercise beneficial for an individual, but it also benefits society. Keeler et al. (1989) demonstrated that individuals with a sedentary lifestyle actually cost society due to subsidized health care costs. In fact, the cost to society of a individual's sedentary lifestyle is nearly double that of cigarette smoking (\$1,900 versus \$1000) (Keeler et al. 1989, Manning et al. 1989).

Despite all the evidence of benefits from exercise and physical fitness, only 22 % of U.S. adults engage in regular sustained leisure time activity for at least 30 minutes 5 or more times a week (U.S. Department of Health and Human Services, 1996). Further, only 14 % of U.S. adults currently engage in regular vigorous leisure time activity (U.S. Department of Health and Human Services, 1996). These percentages are far below the national health objectives for physical fitness and exercise which stated that at least 30 % of people aged 6 years and older should engage in moderate physical activity and 20 % of adults should engage in vigorous physical activity by the year 2000 (U.S. Department of Health and Human Services, 1990a and 1995). Thus, despite strong evidence that exercise is beneficial in promoting health, the data show that the majority of Americans are not exercising.

However, a minority of the population does engage in regular vigorous exercise. For example, 9.1 % of adults in the U.S. engage in running or jogging (Centers for Disease Control and Prevention, 1991).

Many of these runners enjoy competition, as demonstrated by increased participation in local road races (especially 5 and 10 km runs). Recently, longer distance running has gained in popularity among these runners. According to the monthly magazine Runner's World (Hendersen 1992), marathon running has become increasingly popular with recreational runners, who travel to various cities to race. These recreational runners have longer marathon times (over 4 hours) compared to elite runners, yet they still train and accrue heavy mileage.

In order to promote optimal health and well-being, it is necessary to understand the effects of both extremes of exercise on health. While regular exercise is beneficial towards health, excessive exercise is associated with increased risks including illness and injury (Brunet et al. 1990, Nieman et al. 1990, Peters and Bateman 1983). High intensity, long endurance exercise such as marathon running has been shown to suppress immune function (Berk et al. 1990). In particular,

the incidence of upper respiratory tract infections (URTIs) is dramatically increased among marathon and ultramarathon runners prior to and following competition (Nieman et al. 1989, Peters et al. 1983, Peters et al. 1993). This is of great concern to athletes as URTIs may interfere with training and/or competition. In addition, URTIs may result in a loss of work days and more doctor visits and thus is of concern to the medical and insurance industries. To promote optimal health among athletes who train for and engage in long-endurance events and in order to make wise exercise recommendations, we need to determine any contributing or mitigating factors related to risk of URTIs.

Exercise and the Immune System

Exercise is a potential form of both biological and psychological stress and is accompanied by all the hormonal and immunological changes that are associated with psychological stress. Indeed, excessive stress, either psychological or physiological, is known to reduce resistance to various diseases and infections. Psychological stress has been associated with an increase in susceptibility to the common cold (Cohen et al. 1991, Graham et al. 1986). Similarly, prolonged exercise results in an increased risk of URTIs (Heath et al. 1990, Nieman et al. 1990, Peters and Bateman 1983).

Currently, it is unclear what mechanism is responsible for this increased risk of URTIs following heavy training and competition. Most likely, the combination of extreme physical and psychological stress on the body results in the immunosuppression that follows intense prolonged exercise. It is well known that cortisol, produced during stress and prolonged intense exercise, results in immunosuppression (Keast and Morton 1992, Heath 1992). Thus, exercise can be used as a model to study the effects of stress on the immune system and to investigate strategies to improve resistance in stressed individuals.

Endurance exercise results in a transient decrease in natural killer cell activity which has been suggested as a potential mechanism for the increased susceptibility to URTIs during the post-exercise period (Berk et al. 1990). Other potential mechanisms offer somewhat less satisfactory explanations and less research evidence, such as findings of reduced salivary immunoglobulin A levels in athletes (Tomasi et al. 1982) or decreased serum interferon levels (Siegel 1974).

Many athletes and non-athletes alike take vitamin C supplements in an attempt to ward off the common cold. In general, studies of vitamin C supplementation have shown mixed results, generally with little or no decrease in incidence of colds and a consistent but relatively small reduction in the duration of a cold (Chalmers 1975, Hemila 1992). However, with only a couple of exceptions, most of the previous studies of vitamin C were conducted on apparently healthy subjects who presumably were not under excessive psychological or physiological

stress (Anderson 1972, Bendich and Langseth 1995, Hemila 1994). Whether these study results can be generalized to people under severe stress, such as endurance athletes, is questionable. Further, physically active individuals in very warm climates appear to require more vitamin C than the Recommended Dietary Allowance (National Research Council 1989, Strydom et al. 1976, Kotze et al. 1977). Finally, in a recent nutritional intervention study in the elderly, Chandra (1992) found that vitamin and mineral supplements improved various immune system parameters and increased resistance to upper respiratory tract infections in elderly subjects.

Many athletes anecdotally report that they feel that moderate exercise increases their resistance to infection whereas elite and highly competitive athletes report a decrease in resistance around the time of competition (Nieman et al. 1989b and 1990, Nieman 1992, Peters and Bateman 1983). A number of studies have demonstrated that moderate exercise may act as a buffer against infection whereas long-endurance exercise may increase risk (Heath et al. 1991, Nieman et al. 1989 and 1990, Peters et al. 1993). Recently, Peters et al. (1993) found that vitamin C supplements reduced the incidence of URTIs among ultramarathon runners. Thus, while excessive exercise increases the risk of URTIs, important mitigating factors such as vitamin C supplementation, may exist. Possibly, nutritional intervention strategies could be used during intense training and competition to help prevent illness.

Statement of Research Problems

The primary purpose of this study was to determine the effects of vitamin C supplementation on the incidence of URTIs in marathon runners.

A secondary purpose of this study was to identify biomedical parameters that are associated with increased risk of URTIs that are affected by vitamin C supplementation. Blood samples were taken from a subset of the study sample to corroborate the clinical data with biomedical parameters including plasma vitamin C levels and lymphocyte proliferation.

Hypotheses

1. Vitamin C supplementation influences the incidence of URTIs among marathon runners.
2. Vitamin C supplementation affects plasma vitamin C levels in marathon runners and in sedentary subjects over time.
3. Vitamin C supplementation has no effect on lymphocyte proliferation in marathon runners. The null hypothesis has been selected for this hypothesis as the previous literature is unclear.

Sub-hypotheses

1. High stress levels are predictive of URTIs in marathon runners.
2. High training mileage (above 60 miles per week) is predictive of URTIs in marathon runners.
3. Training intensity (faster pace) is predictive of URTIs.

Research Design

A double-blind, placebo-controlled design was used to determine the influence of vitamin C supplementation on the incidence of URTIs among marathon runners and sedentary subjects during the two months prior to and one month following the Duke City Marathon in Albuquerque, N.M on September 11, 1994.

Statistical Design

The Statistical Analysis System (SAS Institute, Cary, NC) was used to perform the statistical analyses. Incidence (total number of cases) and the percentage of subjects with at least one URTI during the study were determined. Repeated-measures analyses of variance were performed on the plasma vitamin C and lymphocyte proliferation data. Multiple logistic regression was used to determine the factors associated with an increased incidence of URTIs.

Limitations

This study was limited in the following ways:

1. Sample size and dropouts. Two groups of subjects were studied: marathon runners and sedentary subjects. The marathon runners were restricted to those who completed the 1994 Duke City Marathon in Albuquerque, New Mexico. Sedentary subjects were selected from age-matched household members and friends of the runners who engage in no regular aerobic exercise (see definition section) and also volunteers who were recruited on the University of New Mexico campus.
2. Time of the study. This study was limited to the two months prior to and one month following the marathon. This time frame was used because previous findings in the literature indicate marathon runners are particularly susceptible to colds during heavy training and competition (Nieman et al. 1990).
3. Outcome variables. The dependent variables were limited to plasma vitamin C levels, lymphocyte proliferation, and incidence of URTIs.

Delimitations

Factors which were investigated but not controlled in this study include:

1. Estimated usual dietary intake of vitamin C in the year before the study.
2. Supplemental intake of vitamin C prior to the study.
2. Stress level.
3. Training mileage.

Assumptions

1. Study subjects took the vitamin C or placebo tablet daily throughout the intervention period. This assumption was verified in part by having the subjects return the containers of vitamin C or placebo at the end of the study and performing a pill count for each subject. Further, the subjects were asked in person and by questionnaire if they took the vitamin C or placebo tablet daily throughout the study. In addition, plasma vitamin C levels were determined on a subset of the study population.
2. Sedentary subjects remained inactive during the study period. This assumption was confirmed on a subset of the study sample by questioning them during the second visit to the Clinical Research Center to determine exercise activity.
3. Incentives were used to help retain study subjects who had blood samples drawn. Only those subjects who were willing to submit to a blood drawing over the course of the study were recruited into this part of the study. In addition, total cholesterol levels and body-fat testing were determined largely as an incentive for volunteering to provide blood samples.

Incentives, such as coupons for free running shorts or singlets and discounts on running shoes at local sporting good stores, were also used to help retain study subjects as this study required a three-month commitment to take the vitamin C or placebo treatment daily and fill out and return questionnaires.

Chapter Two Review of the Literature

"Vitamins, if properly understood and applied, will help us to reduce human suffering to an extent which the most fantastic mind would fail to imagine." (Szent-Gyorgi 1937).

Exercise and URTI Incidence

Recently, there has been a surge in interest in the effect of exercise on the immune system. Two population groups have been studied extensively, the elderly and athletes. A number of investigators have reported health benefits, particularly an increased resistance to upper respiratory tract infections (URTIs), among those who engage in moderate exercise. Clinical trials have been conducted to assess the impact of exercise on resistance to URTIs among the elderly (Karper and Boschen 1993, Nieman et al. 1993) and among premenopausal women (Nieman et al. 1990).

Karper and Boschen (1993) studied 14 subjects who were between 60 and 72 years old. They reported that a walking program resulted in a reduction in upper and lower respiratory tract infections in 11 out of 12 subjects compared to the previous year. In addition, 8 out of 14 subjects reported reduced stress levels during the exercise program. However, no control group was studied.

Nieman et al. (1993) randomly assigned exercise treatments to a group of previously sedentary elderly women aged 67 to 85 years old in a 12-week study. Fourteen women participated in a walking (experimental) group and 16 women were in the calisthenic (control) group. In addition, 12 highly conditioned elderly women who participated in local races were studied. The highly conditioned women had superior immune function as determined by increased natural killer and T-cell function compared to sedentary women. Incidence of URTIs was lowest in the highly conditioned women (8%), moderate in the walking group (22%), and highest in the calisthenic group (50%) during the 12 week study.

Nieman et al. (1990) also examined the effect of a walking program on URTIs among premenopausal women. Thirty-six overweight premenopausal women were randomized into either a control group or a walking group. Walkers engaged in a 45 minute brisk walking session five times per week for 15 weeks. The exercise program resulted in a 20% increase in serum immunoglobulins and a 57% increase in natural killer cell activity. Although no difference was found in incidence of URTIs, subjects reported fewer URTI symptom days (10.8 symptom days among the sedentary women compared to 5.1 among the walkers).

Alterations in immune status have also been reported for runners and for cross country skiers (Nieman et al. 1995, Nieman 1992, Tomasi et al. 1982). Heath et al. (1991) examined risk factors for URTIs in an epidemiological survey of 530 runners over a 12-month period using self report data. Runners were recruited by mail using a mailing list of 1,573 potential runners who requested information on road races in South Carolina. Each potential subject was sent a baseline questionnaire and informed consent form, and upon the return of these was then sent a monthly exercise and health status log. Of the 966 runners who returned the baseline questionnaire and consent form, 530

(55%) completed the monthly logs for all 12 months. Analysis of dropouts showed that they were similar to runners who completed all 12 months of the study.

There were 447 (84%) men and 83 (16%) women in the study. The 530 runners kept detailed exercise and health status logs. URTIs were assessed by symptoms of runny nose, sore throat, or cough on two consecutive days that was not due to allergy or chronic illness. Potential risk factors for URTIs that were examined included number of days run per week, mileage run per day and per year, racing history, and marathon participation. Annual mileage was examined in quartiles, with the lowest quartile being used as the reference group. In addition, the effects of age, gender, body mass index, marital status, smoking status, alcohol use, breakfast habits, vitamin use, sleep, number of people in the home, and days of school or work missed due to illness were examined.

Data were analyzed using descriptive statistics and by comparing subjects with and without URTIs using chi-square tests and logistic regression. Odds ratios of risk for developing URTIs were determined by logistic regression and backward stepwise logistic regression.

The average number of URTIs per subject per year was 1.2. Using a multiple logistic regression model, the following factors were found to be associated with an increased risk of URTIs: living alone (odds ratio = 2.27, 95% confidence interval (CI) = 1.0, 5.1), running mileage (784-1395 km, odds ratio = 2.0, 95% CI = 1.0, 2.8; 1397-2239 km, odds ratio = 3.5, 95% CI = 1.5, 4.44; above 2239 km, odds ratio = 3.0, 95% CI = 1.3, 3.7). Body mass index above the 75th percentile and male gender were both associated with a lower risk of URTIs (odds ratio = 0.6, 95% CI = 0.3, 0.9 and odds ratio = 0.1, 95% CI = 0.0, 0.7 respectively). The greatest risk of URTIs was found in subjects who ran in the third quartile, or 1397-2239 km (866-1388 miles) per year. These runners had a 3.5 times increased risk of URTIs compared to subjects who ran under 784 km (486 miles) per year. In addition, runners in the highest quartile had about a 3 times increased risk of URTIs compared to low mileage runners. The authors concluded that annual running mileage above 784 km (486 miles) per year was a significant risk factor for URTIs.

This well-designed prospective study with a large number of subjects enabled the researchers to examine a broad range of variables that might be related to risk of URTIs. The study would probably have been more powerful had they had a true non-running control group. In addition, body composition was assessed indirectly with body mass index.

URTIs Before and After a Road-race

Nieman et al. (1989a) assessed the incidence of URTI in runners during a two-month period (January and February) prior to and in the one week following a road-race (5 km, 10 km, or half-marathon). The road-race "Run Thorough Redlands" took place on March 9, 1986 in Redlands, California.

Questionnaires were sent to 510 randomly selected participants out of approximately 1200 total participants. Questionnaire data included demographics, training habits, race performances, history of injuries, and illnesses in the runners and in housemates for the two-month period prior to and the one week period following the race.

Running data were analyzed by race category and by weekly training mileage (less than 24 km or 24 km and above). A one-way analysis of variance was performed to compare training differences among race categories. A student t-test was used to compare mileage groups. Chi-square tests were used to determine differences between sick or injured runners by race or mileage categories.

Of the 510 questionnaires sent out, 294 were returned (58%). Twenty-one subjects were excluded due to not participating in the race or for lack of complete information. Non-responders were surveyed and found to be less involved in their training than responders. Of the total 273 subjects, 183 were male and 90 were female.

Thirty percent of the runners (not separated by race category) reported one or more URTIs during the two months prior to the race. No differences were found between males and females in the incidence of URTIs.

When the runners were compared by race category, 31.6%, 30.4%, and 22.7% of the runners in the 5 km, 10 km, and half marathon, respectively, reported one or more URTIs during the two-month period prior to the race. The researchers then combined the 5 km and 10 km runners and found that 31% reported at least one URTI compared to 22.7% of the half marathoners. Only 6.8% of the half marathoners reported at least one flu compared to 17.9% of the combined 5 km and 10 km runners during the two-month period.

A more than two-fold increase was found in reported cases of at least one sick housemate among the runners who had URTIs. However, no significant differences were found in the number of cases of sick housemates between race categories. Of the runners who reported at least one sick housemate, there was a higher incidence of URTIs among the half-marathoners and 10 km runners, compared to the 5 km runners (54.3%, 42.1%, and 29.4%, respectively).

When the data were analyzed by running mileage, 25% of the runners in the 24 km (15 miles) per week or higher category reported one or more URTIs during the two-month period, compared to 34.3% of the lower distance runners ($p=0.09$; a trend, but not statistically significant).

No differences were found in the rate of URTIs in the week after the race compared to the week prior to the race.

The authors concluded that runners who trained more than 24 km (15 miles) per week had fewer URTIs compared to those who trained less than 24 km per week. The researchers speculated that this may be due to alterations in lymphocyte function and natural killer cell activity following moderate submaximal exercise.

However, the researchers cautioned that this study was done on a self-selected group. In addition, the improved resistance to URTIs may not be directly caused by the exercise, but could be due to some mechanism whereby exercise results in a reduction in stress.

This is an interesting study of the impact of varying levels of exercise on the incidence of URTIs. However, the cutoff of running above or below 24 km (15 miles) per week is rather low given that most competitive athletes and many fitness enthusiasts engage in much higher levels of activity. By studying only two groups of runners who averaged 12 km (7.5 miles) versus 42 km (26 miles) per week, the study results are limited and cannot determine the effects of longer distance running on resistance to URTIs.

A second problem with this study is that while the authors reported differences between categories of training and incidence of URTIs, the results were not statistically significant. Possibly, a larger sample size would have resulted in significant differences (only 44 half-marathoners were included in the study).

In addition, there was no clear rationale other than the small sample size to put the 5 and 10 km runners into one category as there were differences between these two groups in weekly training distances and in illness rate among those runners with at least one sick housemate. The lower incidence of URTIs among 10 km runners suggests a protective effect of higher training mileage between 5 and 10 km runners, perhaps due to some protective effect of exercise.

Of interest, Heath et al. (1991) found a lower risk of URTIs among males compared to females using a regression model (odds ratio = 0.6, CI = 0.3, 1.0), although the mean incidence was similar at 1.2 and 1.3 URTIs for males and females per year. In contrast, Nieman et al. (1991) reported no significant difference in sickness between males and females, with 29% of males and 31% of females reporting URTIs during the two months prior to the marathon. Heath et al. (1991) found a slightly higher risk of URTIs among runners who ran 784-1395 km (the equivalent of 15-27 km per week) per year, compared to those who ran under 784 km per year (15 km per week), which is contrary to the findings by Nieman et al. (1989a). Further, Heath et al. (1991) reported an even greater incidence of URTIs among runners with larger annual mileage. These findings suggest that the cutoff of 24 km (15 miles) per week in the Nieman et al. study (1989a) may have been set too low, and that a continuous analytical model may have been preferable.

URTIs Before and After a Marathon

Nieman et al. (1990) theorized that high mileage training and high levels of perceived stress would increase the risk of URTIs among marathon runners who participated in the 1987 Los Angeles Marathon.

Runners were randomly recruited eight days before the marathon using a data base of all race participants. Questionnaires and return envelopes were sent to each subject and were received approximately one week following the marathon.

The authors used a revised version of their questionnaire from the 1989 study (Nieman et al. 1989a). Data collected included training habits, race results, and incidence of URTIs among runners and among members of their households.

Runners were asked to report average weekly training distance for 1986 and for the two month period during the winter months prior to the marathon (January and February, 1987). In addition, runners reported their longest weekly runs and the intensity of these runs using a 10-point rating of perceived exertion. Incidence of URTIs was reported for the two month period prior to and the one week period following the March 1, 1987 Los Angeles Marathon. In addition, symptoms and duration for each URTI were reported by the subjects.

Stress levels (high versus low) were determined from four-point Likert scale questions about sleep, energy, stress levels, and overall feelings during the running years compared to non-running years.

Statistical methods included multivariate analyses using a logistic regression model to ensure that results were not due to differences in demographics. Multivariate analysis of variance was used to determine differences between runners who reported URTIs from those who did not. Runners were divided into 6 categories based on weekly training distance for data analysis. Odds ratios for acquiring URTIs were calculated for runners for the two months prior to and one week following the marathon.

Of the initial 4926 questionnaires sent to race applicants, 2311 were returned (46.9%). A total of 10,759 runners finished the marathon; 2016 of these were included in the study. Another 295 race applicants returned the questionnaire but did not finish the race; all study subjects were encouraged to return the questionnaire whether or not they completed the marathon. The researchers compared the study sample of finishers to other finishers who were not included in the study and found that the study sample was slightly older (mean \pm SEM = 36.9 ± 0.2 versus 34.9 ± 0.1 , $p < .01$). No significant difference was found between the study sample and other finishers in the proportion of males versus females (85.0% male, 15.0% female versus 86.2% male, 13.8% female). No differences were found in training statistics between men and women; hence, both sexes were combined for data analysis.

The majority of the study sample reported feeling lower levels of stress since starting to run, as well as higher energy levels and better handling of stress.

Only 36.3% of runners who were in the low stress group reported URTIs compared to 45.2% of runners in the high stress group (odds ratio = 1.4, 95% CI = 1.2-1.7). Interestingly, more of the high distance runners were classified in the high stress group.

Average training mileage was 61.0 ± 0.6 km ($37.8 \pm .37$ miles) (mean \pm SEM) per week during the two month period prior to the marathon. Only 10.0% of the runners averaged above 97 km (60 miles) per week. The average runner increased training distance during this period by about 15.6 ± 0.5 km (mean \pm SEM) (10 miles) per week compared to average training during 1986.

During the two month period prior to the marathon, 43.2% of all the study subjects reported one or more URTIs. Odds ratios for acquiring URTIs for runners training more than 97 km (60 miles) per week versus less than 32 km (20 miles) per week was 2.0 (95% CI 1.2-3.4). However, no differences were found among the runners in the five

lower mileage groups when the runners over 97 km (60 miles) per week were omitted from the analysis.

During the week following the marathon, 236 of the 1828 study subjects (12.9%) reported a URTI. Only runners who were free of URTIs during the week before the marathon were included in this analysis (342 runners became sick during the week before the marathon; all but 34 ran the marathon). Of the 134 runners who were not ill but chose not to run the race, only 3 (2.2%) became ill during the one week period after the marathon. Thus, the odds ratio for acquiring a URTI for race participation was 5.9 (95% CI 1.9-18.8). However, nonparticipants trained less prior to the marathon compared to the marathon finishers (nonparticipants ran about 44 km (27 miles) per week compared to finishers who ran 63 km (39 miles) per week during the two months prior to the race). The authors reported that entering training distance into the regression model did not alter the effect of participating in the marathon on risk of URTIs.

In summary, the authors reported that the runners had an increased risk of URTIs during heavy training and following the marathon. Runners who trained over 97 km (60 miles) a week had a two-fold increase in their risk of URTIs compared to those who trained 32 km (20 miles) or less per week. In addition, 12.9% of the runners who were healthy prior to the race reported URTIs during the week following the marathon compared to only 2.2% of similar runners who did not race.

However, the average training distance of the study sample was only about 61 km (38 miles) per week during the two month period prior to the marathon. This mileage is rather low for marathon runners as many elite and highly competitive runners average 97 km (60 miles) per week or more during training. This limits the generalizability of the study results to marathoners who train at lower weekly distances.

In addition, the researchers did not report where runners lived. Typically, a large number of runners travel to the Los Angeles area for the marathon each year and those from colder climates or who had to fly to Los Angeles might have been at greater risk of URTIs. Also, occupations of the runners were not reported. Certainly people who work among children would be at increased risk of URTIs.

The study sample was selected eight days prior to the marathon and received the questionnaire approximately one week following the marathon. Thus, the data collection relied on subject memory over the past two months and may have introduced bias. For instance, subjects who had repeated URTIs which interfered with their training would be the most likely to report URTIs among themselves and members of their households. A better study design would be to recruit and collect the data prospectively to avoid memory bias.

The authors reported that the low stress group had a lower incidence of URTIs. However, the high stress group also had the highest mileage. Thus, it is not clear whether stress levels or high mileage or an interaction of both were responsible for the increased incidence of URTIs. Further, reliability and validity for the questionnaire items on stress were not reported. The data on current

stress levels compared to the levels prior to running relied on memory and could be biased (on average, the runners reported 7.2 years of running experience).

Finally, the authors used race applicants who did not run the marathon as a control group for comparison to examine the risk of acquiring URTIs during the week following the marathon. Nonrunners and runners had similar incidence of URTIs during the two months prior to the marathon. However, the nonrunners trained less during the two months prior to the marathon which makes them a poor choice for control group. Although the authors claimed that they accounted for the differences in training in the regression model, the fact that the group trained less would lead one to speculate that they would be at decreased risk of URTIs based on the authors' conclusions from the study. Also, runners who traveled to the marathon may have had more exposure to airplanes, crowds, etc. which may have increased their risk of acquiring URTIs, unlike the nonrunners who may have remained at home.

On the positive side, the researchers compared demographic data of marathon runners to nonrunners and to nonresponders to assure that there were not large differences between these groups which would limit the generalizability of the study findings. Finally, the study included a large number of subjects and had a good response rate.

Overview of the Immune System

This section reviews the main components of the immune system, with an emphasis on aspects that are affected by exercise or vitamin C.

The purpose of the overview is to introduce terminology for the following sections of the literature review. (See Definitions in Appendix). A brief discussion of the organization and cellular components of the immune system follows below.

The immune system is comprised of a complex network of specialized cells and organs which act to defend the body against foreign invaders such as bacteria and viruses. The two types of immunity are humoral and cell-mediated. Humoral immunity refers to the secretion of soluble substances into body fluids such as antibodies which bind to circulating antigens. Cell-mediated immunity refers to the direct action of immune cells such as natural killer cells and cytotoxic T-cells with target cells.

Lymphocytes are small white blood cells which play an important role in immunity. B-cells and T-cells make up the two major classes of lymphocytes and together number about 10^{12} (US Department of Health and Human Services 1990). B-cells mature in bone marrow whereas T-cells mature in the thymus, a lymphoid organ in the chest. B-cells secrete antibodies which provide for humoral immunity, while T-cells interact directly with target substances providing cellular immunity.

B-cells differentiate into plasma cells that produce immunoglobulins or antibodies, which can be of the following types: IgG, IgM, IgA, IgE, and IgD. The major immunoglobulin in the blood is IgG, which can also enter into tissue spaces. IgM is present only in the blood. Tears, saliva, respiratory, and intestinal secretions

contain IgA. IgE plays a role in the protection from parasites and in the development of allergies. IgD is present on the membranes of B-cells.

There are three classes of T-cells: helper T-cells, suppressor T-cells, and cytotoxic T-cells. Helper T-cells produce IL-2, IL-4, and IL-5 (see below). Cytotoxic T-cells are capable of destroying any cell with a foreign or abnormal antigen. However, cytotoxic T-cells must first recognize the antigen on the target organism before they can kill it, unlike natural killer (NK) cells discussed below. Suppressor T-cells oppose the actions of helper T-cells and can turn off antibody production and immune responses.

Both T and B-cells secrete lymphokines which are involved in inflammation, cell growth, macrophage activity, and destruction of target cells. For example, gamma interferon is a lymphokine produced by T-cells which has antiviral activity and activates macrophages. Other lymphokines include the interleukins such as IL-1 and IL-2. IL-1 is produced by macrophages and activates B-cells and T-cells. IL-2 is produced by activated T-helper cells and promotes growth and differentiation of B-cells and T-cells. NK cells are lymphocytes which are responsible for immune surveillance and can react to foreign bodies without antibody or antigen recognition. NK cells have granules filled with potent chemicals. In addition, NK cells secrete lymphokines, including gamma interferon.

Interferon refers to a heterogeneous group of cytokines which have antiviral activity and stimulate the immune system. Alpha- and beta-interferon are produced by virally infected cells and function by stimulating NK and macrophage activity. Gamma-interferon is produced by activated helper T-cells and NK cells and exerts weak antiviral activity and activates many immune cells.

Phagocytes (neutrophils and monocytes) are large white blood cells which can engulf and digest foreign substances. For example, monocytes are mononuclear phagocytic cells present in the blood which can differentiate into macrophages in various tissues. Granulocytes are a heterogeneous group of white blood cells which have multi-lobed nuclei and intracellular granules. Examples of granulocytes include eosinophils, basophils, and neutrophils. Eosinophils play a role in defense from parasites and in the reduction of inflammation. Basophils contribute to the inflammatory response and to allergy symptoms. Neutrophils are phagocytic granulocytes.

Complement is a series of 9 proteins (C1, C2, C3, etc.) that assists antibodies in the destruction of bacteria, aids in the removal of the antibody-antigen complexes, and is involved in inflammation.

Exercise and the Immune System

While moderate exercise appears to enhance resistance to disease, probably by increasing NK cell activity, intense prolonged exercise negatively impacts resistance, possibly through a transient decrease in NK cell activity post-exercise (Berk et al. 1990). The decrease in NK

cell activity may be mediated by the increased cortisol levels which occur in prolonged intense exercise.

In addition, various other immune parameters, such as reduced serum or salivary immunoglobulin levels (Tomasi et al. 1982) and reduced interferon levels (Siegel 1974, Reyes and Lerner 1976, reviewed in MacKinnon 1992), have also been proposed as mechanisms for the exercise-induced immunosuppression. All three types of interferon discussed above are known to stimulate NK cells (MacKinnon 1992). Although information on the effects of exercise on interferon is lacking, one study found that forced exercise suppressed the release of interferon in mice (Reyes and Lerner 1976). However, Viti et al. (1985) reported that one hour of cycling at 70% of maximum oxygen consumption resulted in an increase of alpha-interferon by 100% immediately post-exercise and at one hour, but no difference at two hours post-exercise in four male cyclists. Haahr et al. (1991) found no change in gamma-interferon levels with exercise. The increase of alpha-interferon with exercise stimulates NK and macrophage activity and may be related to the enhancement of immune function that occurs with moderate exercise (Mackinnon 1992). The effects of moderate and prolonged exercise on the immune system are summarized on Tables 1 and 2.

Janssen et al. (1989) studied changes in white blood cells associated with marathon training in 60 males and 18 females who were previously untrained over an 18- to 20-month period. White blood cells were measured at baseline and one week before and after three road races (15, 25, and 42 km). No changes were found in the number of leukocytes, lymphocytes, or neutrophils one week following each of the road races. In addition, no change occurred due to training in leukocyte or neutrophil number. However, the number of eosinophils, basophils, and monocytes was increased one week following the road races, particularly with the longer distance race. Following the marathon (42 km), there was a greater increase in leukocytes in females than in males, primarily due to an increase in neutrophils; however, this result was not statistically significant.

Scavo et al. (1991) studied adrenocorticotrophic hormone (ACTH), beta-endorphin, cortisol, growth hormone, and prolactin levels in nineteen athletes before and after a half-marathon and marathon in Rome. Blood samples were drawn 48 hours and 1 hour prior and between 10 and 20 minutes following each race. ACTH, beta-endorphin, and cortisol levels were significantly elevated in marathon runners 1 hour prior to the race, presumably due to stress. In addition, the hormonal increases following the race were higher in marathon runners compared to half-marathon runners. The authors concluded that the hormonal changes were affected by the duration of the race.

Berk et al. (1990) investigated the effect of marathon running on NK cell activity and number in trained marathoners. Ten marathon runners (nine males and one female) were studied in the laboratory. Blood samples were taken at rest, at 1 hour of exercise, and at 5 minutes, 1.5 hours, 6 hours, and 21 hours of recovery. NK cell activity was decreased at 1.5 and 6 hours of recovery, but returned to

baseline values by 21 hours. Post-exercise cortisol (at 5 minutes of recovery) was inversely related to NK cell activity at 1.5 hours of recovery ($r = 0.62$, $p < 0.05$). The authors suggested that cortisol may cause a reduction in lymphocyte numbers temporarily by keeping helper T-cells in the bone marrow and by inhibiting the release of IL-1, which is needed to induce release of IL-2, which causes lymphocyte proliferation. Further, cortisol may reduce NK cell activity by altering interferon levels. Berk et al. (1990) suggested that reduced NK cell activity following marathon running may increase susceptibility to URTIs.

Nieman et al. (1989b) examined the effect of marathon running on various immune system parameters in the same ten marathoners described above (Berk et al. 1990). This report evaluated changes in leukocytes, lymphocytes, lymphocyte stimulation (spontaneous blastogenesis), cortisol, and catecholamines during the 3-hour laboratory run.

Lymphocyte count increased 31% at 1 hour of exercise, but then a mild lymphopenia occurred during recovery. Significant leukocytosis, granulocytosis, neutrophilia, monocytosis, and eosinopenia occurred during recovery but returned to normal within 21 hours except for eosinophils. Cortisol levels increased between baseline and 1.5 hours of recovery and were correlated with increased leukocytes ($r = 0.78$, $p < 0.008$) and granulocytes ($r = 0.81$, $p < 0.005$). T-helper to T-suppressor cell ratio was increased 39% at 1.5 and 21 hours of recovery due to a decrease in T-suppressor cell number. Lymphocyte stimulation increased 53% after 1 hour of exercise and was elevated during recovery. The authors suggested that the increase in lymphocyte stimulation and T-helper to T-suppressor ratio may be reflective of an overall improvement in immunity, whereas the temporary decrease in NK cell activity (Berk et al. 1990) may acutely compromise cell-mediated immunity in marathon runners.

Recently, Nieman et al. (1995) compared immune function in a group of experienced marathon runners ($n = 22$) to sedentary subjects ($n = 18$). Neutrophil counts were slightly lower in the marathoners (mean \pm SEM = 2.66 ± 0.20) versus sedentary subjects (3.29 ± 0.27) ($p < 0.06$) (units = 10^9 per liter). Concanavalin A (Con A) and phytohemagglutinin (PHA)-induced lymphocyte proliferation did not differ between marathoners and sedentary subjects. Natural killer cell number was similar between marathoners (0.35 ± 0.06) (mean \pm SEM) and sedentary subjects (0.31 ± 0.04) ($p < 0.58$) (units = 10^9 per liter). However, natural killer cell activity was 57% higher in the marathon runners compared to sedentary subjects ($p < 0.05$) and was negatively correlated with percent body fat. In addition, age was negatively correlated with Con A-induced lymphocyte proliferation. The authors concluded that natural killer cell activity was higher in marathon runners whereas lymphocyte proliferation was similar to sedentary subjects.

TABLE 1. Summary of the Effects of
Moderate Exercise on the Immune System

- Increased natural killer activity in elderly walkers and runners (Nieman et al. 1990, 1993)
- Improved T-cell function in elderly walkers and runners (Nieman et al. 1993)
- Increased serum immunoglobulins in obese premenopausal women (Nieman et al. 1990)
- Increased alpha-interferon at one hour of cycling (Viti et al. 1985)
- Decreased risk of upper respiratory tract infections in walkers and runners (Karper and Boschen 1993, Nieman et al. 1989a, 1990)

TABLE 2. Summary of the Effects of
Prolonged Exercise on the Immune System

- Decreased
NK activity in marathon runners (Berk et al. 1990)
 - Increased cortisol in marathon runners (Scavo et al. 1991, Berk et al. 1990, Nieman et al. 1989b)
 - Decreased salivary and serum immunoglobulins in cross-country skiers (Tomasi et al. 1982)
 - Increased eosinophils, basophils, monocytes in marathon runners (Janssen et al. 1989)
 - Increased beta-endorphin, adrenocorticotropin hormone (ACTH) in marathon runners (Scavo et al. 1991)
 - Decreased lymphocytes and eosinophils, increased leukocytes, granulocytes, monocytes, and neutrophils in marathon runners (Nieman 1989b)
 - Increased T-helper to suppressor ratio (Nieman 1989b)
 - Increased lymphocyte stimulation (Nieman 1989b)
 - Increased risk of upper respiratory tract infections (Heath et al. 1991, Nieman et al. 1990, Peters et al. 1983, 1990)
-

Vitamin C and the Immune System

"The literature in this field is bedeviled by controversy and lack of confirmation" (Thomas and Holt 1978)

During physiological or emotional stress, cortisol is released from the adrenal cortex in response to ACTH from the pituitary gland. Large amounts of vitamin C are also released from the adrenal cortex during stress, prior to the release of cortisol. Vitamin C levels have been found to be increased in the blood during stress and during prolonged exercise (Fishbaine and Butterfield 1984, Garry and Appenzeller 1983, Maxwell 1993).

Cortisol is known to be immunosuppressive. In contrast, vitamin C appears to enhance immune function and large doses (2 gm) can correct some of the corticosteroid-induced effects on polymorphonuclear cell function in patients on long-term steroid treatment (Chretien et al. 1973).

Vitamin C and the Immune System: Macrophage Migration

Guinea pigs have been used in a number of studies of vitamin C deficiency as they are one of few mammals besides the human that cannot synthesize vitamin C. The effects of vitamin C deficiency on the immune system are summarized in Table 3. Vitamin C deficiency has been shown to result in decreased migration of macrophages in guinea pigs (Gangule et al. 1976). Vitamin C deficiency in guinea pigs was shown to interfere with the bactericidal action of leukocytes compared to vitamin C replete leukocytes to the oral pathogen Actinomyces viscosus (13% versus 83% killing of bacterial cells) (Goldschmidt et al. 1988).

Chemotactic responses were also lower in vivo and absent in vitro in vitamin C deficient leukocytes.

Vitamin C and the Immune System: Neutrophil Function

Large doses of vitamin C may improve neutrophil function through a variety of mechanisms, including increased bactericidal action, increased phagocytic activity, and protection against cell membrane oxidative damage (Leibovitz and Siegel 1977, Thomas and Holt 1978, Beisel 1982, Anderson and Theron 1990). (The effects of vitamin C supplementation on the immune system are summarized in Table 4). Anderson et al. (1980) found that 2 and 3 gm of vitamin C daily stimulated neutrophil chemotaxis and motility whereas no effect was seen with 1 gm per day. However, this study had a small sample size and only used one leukoattractant.

Vitamin C and the Immune System: Free Radicals, Lymphocyte Proliferation

Vitamin C may act by neutralizing free radicals formed during the respiratory burst of phagocytic cells (Anderson and Theron 1990).

Vitamin C is also involved in various other immune functions, including lymphocyte proliferation and migration and in histamine degradation. Fraser et al. (1978) reported that vitamin C deficiency

resulted in an increased ratio of B to total lymphocytes in immunized male guinea pigs. In addition, vitamin C deficiency resulted in reduced lymphocyte proliferation with and without the mitogens concanavalin A (Con A) and phytohemagglutinin (PHA) (1.0 ug Con A or 1.5 ug PHA added to 10 ul medium) compared to guinea pigs fed 250 mg sodium ascorbate per day in the 28 day study.

Anderson et al. (1980) found that vitamin C ingestion by human volunteers resulted in increased T-lymphocyte reactivity to the mitogens PHA and Con A at 25 and 50 ug/ml concentrations following 1, 2, and 3 gm of vitamin C per day. Increased lymphocyte transformation occurred with 1, 2, and 3 gm of vitamin C per day whereas protein synthesis was unaffected. Similarly, Kennes et al. (1983) reported that 500 mg/day of injected vitamin C improved T lymphocyte response to mitogens in vitro. Delafuente et al. (1986) reported that vitamin C enhanced Con A-mediated lymphocyte proliferation when added in vitro to samples from healthy subjects, but had no effect when subjects ingested 10 gm of vitamin C per day. Jacob et al. (1991) found no effect of vitamin C depletion on in vitro proliferation of peripheral blood mononuclear leukocytes in healthy men using PHA and Con A (10 ug/ml). Similarly, Kay et al. (1982) reported no effect of vitamin C deficiency on T-cell responsiveness to mitogens.

Delafuente et al. (1986) suggested that the discrepancies between studies of vitamin C and lymphocyte proliferation might be due to the use of different mitogens or different doses of vitamin C or to studying the subjects at different times between studies. For example, PHA and Con A activate primarily T-lymphocytes, whereas lipopolysaccharides activate B-lymphocytes and pokeweed tends to activate both T and B-lymphocytes (Shive 1988).

Vitamin C and the Immune System: Immunoglobulin and Complement Production

Prinz et al. (1977) reported a small but significant increase in serum immunoglobulins (IgA and IgM) and the complement component C3 in 25 subjects who were supplemented with 1 gm of vitamin C daily for 75 days. In contrast, Anderson et al. (1980) found no effect of 1, 2, and 3 gm per day vitamin C in a 3 week study using progressively higher doses on serum levels of IgG, IgA, IgM, C3, C4, or total complement activity. The authors suggested that the shorter supplementation period may have contributed to the different findings compared to Prinz et al. (1977). Anderson et al. (1980b) also found that vitamin C had no effect on salivary IgA levels in the same subjects. However, they noted that the study was performed on healthy subjects whereas salivary IgA levels are dependent on antigen stimulation. Prinz et al. (1980) reported that 160 mg per day of vitamin C in drinking water stimulated IgM production in guinea pigs following immunization to sheep red blood cells.

Sakamoto et al. (1981) reported altered levels of complement components in scorbutic guinea pigs (C1, C2, C3, and C4) in an 8 week experiment. During the first week, all the complement components except C1 decreased, although only C2 was significant. Later, C1

varied during the study while C2 and C3 increased and C4 remained unchanged. Feigen et al. (1982) also found no effect of a large dose (280 mg) of vitamin C per day given parenterally to guinea pigs on complement activity. However, they did find that humoral antibody response was enhanced by vitamin C. Thus, vitamin C does not appear to play a major role in complement and mixed results have been reported for immunoglobulin production (Prinz et al. 1977, Anderson et al. 1980).

Vitamin C and the Immune System: Interferon and Natural Killer Cell Activity

Other researchers have reported increased interferon levels with vitamin C supplementation (Siegel 1974, Dahl and Degre 1976). Interferon is known to stimulate NK cells (MacKinnon 1992) and vitamin C has been shown to enhance interferon response to a viral infection in mice (murine leukemia virus) (Siegel 1974). In addition, Dahl and Degre (1976) found that vitamin C enhanced interferon production in human cells induced by Newcastle disease virus in vitro.

Interestingly, Siegel and Morton (1983) found no effect on NK cell activity in mice supplemented with 250 mg percent vitamin C in their drinking water. The authors pointed out that previous studies which showed an enhancement of interferon levels with vitamin C supplementation used exogenous inducers along with vitamin C. Further, other factors such as the age of the mice can affect NK cell activity, with maximal activity occurring between 3 and 12 weeks of age (Siegel and Morton 1983).

Vitamin C and the Immune System: Antioxidant Role

While the exact biochemical mechanism by which vitamin C enhances immune function has not been established, it is probably related to the antioxidant role of vitamin C (Hemila 1992, Anderson 1984, Thomas and Holt 1978). During infection, neutrophils become activated and produce oxidizing agents which are then released. Extracellular vitamin C can react with these substances and prevent the immune cell from becoming damaged or releasing oxidizing agents. Thus, extracellular vitamin C may be important in protecting neutrophils from autoxidation of the cell membrane and thereby enhance neutrophil mobility and function (Anderson 1984). This would potentially help reduce symptoms and possibly secondary infections of the common cold.

Vitamin C and the Immune System: Hexose Monophosphate Shunt Activity

Other investigators have examined the effect of vitamin C on hexose monophosphate shunt (HMPS) activity (Goetzle et al. 1974, Anderson et al. 1980) and on prostaglandin formation (Manku et al. 1979). Here again, the literature tends not to be very conclusive.

Goetzl et al. (1974) found that 10 to 50 times the usual plasma level of vitamin C increased the migration and chemotactic response of in vitro leukocytes (neutrophils, eosinophils, and mononuclear cells) by 100 to 300 percent, but did not alter phagocytic capacity. In addition, they reported a dose-response relationship between vitamin C

stimulation of HMPS activity and neutrophil mobility. The authors concluded that the immune stimulatory effects of vitamin C appeared to be dependent on leukocyte HMPS activity. In contrast, Anderson et al. (1980) reported that vitamin C had no effect on in vivo post-phagocytic HMPS activity.

Vitamin C and the Immune System: Prostaglandin E₁ Production

Manku et al. (1979) reported that vitamin C caused a dose dependent enhancement of prostaglandin E₁ (PGE₁) production in human platelets. The authors suggest that this regulation of PGE₁ might account for the effects of vitamin C on the immune system such as the enhancement of leukocyte chemotaxis. PGE₁ may also play a role in T-cell maturation and function (Horrobin et al. 1979).

TABLE 3. Summary of the Effects of
Vitamin C Deficiency on the Immune System

-----Decreased
migration of macrophages in guinea pigs (Gangule et al. 1976)

-Decreased bactericidal action of leukocytes in guinea pigs to the oral pathogen Actinomyces viscosus (Goldschmidt et al. 1988)

-Decreased leukocyte chemotaxis in vivo and absent in vitro in guinea pigs (Goldschmidt et al. 1980)

-Decreased lymphocyte proliferation in guinea pigs (Fraser et al. 1978)

-Increased ratio of B to total lymphocytes in guinea pigs (Fraser et al. 1978)

 TABLE 4. Summary of the Effects of
 Vitamin C Supplementation on the Immune System

-----Correction
 of corticosteroid-induced effects on neutrophil function in
 chronically ill patients (Chretien et al. 1973)

-2 to 3 gm per day increased neutrophil chemotaxis and motility,
 whereas 1 gm had no effect in humans (Anderson et al. 1980)

-1, 2, and 3 gm per day, progressive dose, increased T-cell
 reactivity to mitogens in humans (Anderson et al. 1980)

-500 mg injected daily in elderly subjects for 1 month increased T-cell
 reactivity in vitro but had no effect on IgA, IgG, or IgM (Kennes et
 al. 1983)

-Increased lymphocyte proliferation when added in vitro whereas 2 gm
 ingested per day for 2 weeks had no effect on lymphocyte proliferation
 in chronically ill elderly subjects (Delafuente et al. 1986)

-1 gm per day for 75 days increased serum IgA, IgM, C3 in humans (Prinz
 et al. 1977)

-1, 2, and 3 gm per day, progressive dose, for 21 days had no effect on
 serum IgA, IgG, IgM, C3, C4, total complement activity, or on
 salivary IgA in healthy subjects (Anderson et al. 1980)

-160 mg per day increased IgM in guinea pigs following
 immunization to sheep red blood cells (Prinz et al. 1980)

-100 and 280 mg per day enhanced antibody production in guinea pigs
 (Feigen et al. 1982)

-Increased interferon response to murine leukemia virus in mice fed 250
 mg% vitamin C in their drinking water for 3 months (Siegel 1974)

-Increased interferon production in vitro in human cells exposed to
 Newcastle disease virus (Dahl and Degre 1976)

-Increased hexose monophosphate shunt activity in neutrophils in vitro
 (Goetzl et al. 1974)

-Increased PGE₁ in human platelets (Manku et al. 1979)

-Reduced histamine levels in humans with 2 gm per day but no effect
 with 500 mg per day (Johnston et al. 1992)

Vitamin C and the Common Cold

Vitamin C has been proposed as a therapeutic agent against the common cold (Pauling 1970). However, little evidence supports an effect of vitamin C on the incidence of colds (Chalmers 1975, Hemila 1994, Hemila 1992, Anderson et al. 1980, Beisel 1982). Hemila (1994) recently suggested that vitamin C might play a role in reducing the incidence of colds only in certain subgroups. He pointed out that the only two studies he reviewed that found an effect of vitamin C on incidence were on Canadian military troops during Arctic exercises and ski school students in the Swiss Alps, which may be indicative of an effect of vitamin C during extreme stress conditions (Hemila 1994).

Despite the lack of effects of vitamin C on the incidence of colds, there is a relatively small but consistent effect of vitamin C on reduction of severity and duration of colds. This effect appears to be more pronounced on subjective assessment of cold symptoms than on objective symptoms (Hemila 1992). Most researchers have found that doses as small as 100 mg were as effective as much larger doses (Thomas and Holt, 1978). Typically, a reduction of 20-30% in disability is seen when vitamin C is taken at onset and during a cold (Chalmers 1975, Hemila 1994). However, the efficacy of vitamin C on morbidity varies from study to study (Hemila 1992).

Interestingly, leukocyte vitamin C levels fall dramatically with the onset of a cold and increase back to normal gradually until recovery (Hume and Weyers 1973, Thomas and Holt 1978). Vitamin C appears to be consumed by leukocytes rapidly during infection (Hume and Weyers 1973). Even when supplements are given, it is difficult to achieve normal leukocyte vitamin C levels without extremely high doses (Hume and Weyers 1973). For example, Hume and Weyers (1973) found that 200 mg per day vitamin C failed to increase leukocyte vitamin C levels, whereas 6 gm doses prevented leukocyte vitamin C levels from falling below normal.

Vitamin C Supplementation in Elderly Subjects

Recently, Chandra (1992) reported that vitamin and mineral supplements improved several immune system parameters including the number of T-cells, natural killer cell activity, lymphocyte response to PHA, IL-2 production, and antibody response, and significantly decreased respiratory tract infections among 96 independently-living elderly subjects over the course of one year. Nutrient deficiencies in vitamins A, B6, C, and iron and zinc (defined as blood concentrations below the 95% confidence limits of a healthy elderly control group) were corrected by the supplement. In contrast, another group of researchers found that a multivitamin-mineral supplement taken daily for four months resulted in no differences in the incidence of infections per questionnaires in 204 non-institutionalized elderly subjects (Chavance et al. 1992). Possibly, the subjects were not as deficient in this study since average vitamin C intake was above the RDA. In addition, the combination of vitamins and minerals makes any attempt at determining which nutrient might be responsible for any effect on the immune system difficult. Further, the dosages of vitamin

C in both studies were relatively small, 80 and 90 mg, respectively. Goodwin and Garry (1983) found that megadoses of vitamin C in elderly subjects resulted in a non-significant trend towards increased cell-mediated immunity using skin test reactivity but had no effect on mitogen responses. Subjects who had the top 10% of vitamin C intake had the lowest rates of anergy (14%) compared to those with the lower 75% of intake (35%). However, the authors concluded that long-term megadose vitamin supplementation had little effect on immunological function in elderly subjects.

Vitamin C and Pulmonary Function

Vitamin C may act as an antihistamine by assisting in the degradation of histamine (Johnston et al. 1992). Supplements of 1 gm per day have been shown to decrease histamine levels and to improve bronchial constriction in asthmatics (Anderson 1984). However, the results of vitamin C in asthma are mixed and the reported efficacy of vitamin C appears to be rather mild (Anderson 1984, Bucca et al. 1989). Bucca et al. (1989) reported that supplementation of 2 gm per day vitamin C reduced histamine-induced bronchial responsiveness acutely in 9 subjects with URTIs. Johnston et al. (1992) found that 500 mg per day vitamin C had no effect on histamine levels whereas 2 gm per day resulted in about 40% lower histamine levels in two weeks. Hence, relatively large doses are necessary for an antihistamine effect. However, the antihistamine function of vitamin C is unlikely to explain its role in reducing morbidity of the common cold as there is no increase in nasal lavage concentrations during URTIs (Hemila 1992).

A recent study of the relationship between vitamin C intake and pulmonary function in 2526 subjects from the First National Health and Nutrition Examination Survey found that after controlling for a number of factors including age, height, body mass index, race, sex, and cigarette smoking, vitamin C intake was positively correlated with lung function (Schwartz and Weiss 1994). Dietary intake was determined by a 24 hour dietary recall and a food frequency questionnaire. Supplemental intake of vitamin C was not determined. Although the magnitude of the effect of vitamin C on lung function was relatively small, the authors suggested that this could be meaningful physiologically.

Exercise and Vitamin C Status

Physiological stress has been shown to result in decreased plasma and leucocyte ascorbic acid concentrations, which if severe, can approach deficiency levels. Similarly, emotional stress, infection, trauma, and surgery are also known to result in reduced blood levels of vitamin C (Gleeson et al. 1987). The adrenal gland is thought to be the origin of much of the vitamin C in the blood during exercise and stress. Some researchers have speculated that athletes and individuals under severe stress may have an increased requirement for vitamin C (Peters et al. 1993, Goldfarb 1992).

Several studies have shown that vitamin C status and metabolism is altered with prolonged running (Gleeson et al. 1987, Fishbane and Butterfield 1984, Garry and Appenzeller 1983). Blood and lymphocyte vitamin C levels have been shown to increase immediately following prolonged running (Gleeson et al. 1987, Garry and Appenzeller 1983, Fishbane and Butterfield 1984). However, plasma vitamin C levels fall during recovery (Garry and Appenzeller 1983, Boddy et al. 1974) and remain low for at least two days (Gleeson et al. 1987).

Gleeson et al. (1987) studied the effect of exercise on plasma and lymphocyte vitamin C concentration in men who ran a 21 km (13 miles) road race. Nine men aged 26 ± 1 years (mean \pm SEM) participated in the study. All of the subjects were active; however only two ran over 40 km (25 miles) per week during the six months prior to the study. One of the subjects reported taking multivitamins (30 mg of vitamin C) during the previous six months. Blood samples were taken two days prior and one hour prior to the race, within five minutes following the race, and one, two, and three days following the race.

Plasma vitamin C concentrations increased during the race and were positively related to the increase in plasma cortisol levels. Plasma cortisol levels returned to normal within 24 hours of the race. In contrast, plasma vitamin C levels fell beneath pre-race levels at one day post-race and remained decreased at three days following the race.

Although hemodilution of blood plasma accounted for the lower plasma vitamin C level at two and three days following the race, it did not account for all of the reduction at one day following the race (mean \pm SEM = $15 \pm 3\%$ decrease in vitamin C at one day post race after correcting for changes in hemodilution). Lymphocyte vitamin C concentration was increased immediately after the race and remained high at one day post race but returned to normal by the second day.

Garry and Appenzeller (1983) found significantly elevated ascorbic acid levels in 24 men immediately following a 46 km (28.5 mile) mountain run (mean \pm SEM = 1.40 ± 0.09 mg/dl baseline versus 1.62 ± 0.08 finish mg/dl). However, ascorbic acid levels declined at one hour (1.41 ± 0.11 mg/dl baseline versus 1.50 ± 0.08 mg/dl one hour, n=14, not significantly different) and two hours recovery (1.31 ± 0.08 mg/dl baseline versus 1.27 ± 0.08 mg/dl at two hours, n=6, not significantly different).

Fishbane and Butterfield (1984) studied four male runners and seven matched sedentary subjects. Subjects received a controlled diet in a metabolic unit. The runners ran 16 km (10 miles) per day during the first four week period, and 8 km (5 miles) per day for the second four week period. Blood samples were collected during the last day of each period. Serum vitamin C levels were highest following the 16 km run, and lowest in the sedentary subjects (1.50 ± 0.11 mg/dl for 16 km, 1.39 ± 0.16 mg/dl for 8 km, and 1.08 ± 0.05 mg/dl for sedentary subjects)(mean \pm SEM). In addition, 24 hour urinary vitamin C was inversely related to activity level, although this was not significant (98.6 ± 20.4 mg for 16 km, 110.5 ± 43.5 mg for 8 km, and 126.0 ± 12.7 mg for sedentary subjects)(mean \pm SEM). The authors suggest that

physical activity such as prolonged running results in increased metabolic turnover of vitamin C.

In contrast to the findings discussed above, Karnaukh (1976) showed that urinary excretion of vitamin C increased following light exercise in heat. Other researchers have shown that vitamin C needs are increased in severe heat and that vitamin C plays a role in heat acclimatization (Kotze et al. 1977, Strydom et al. 1976).

Robertson et al. (1991) demonstrated alterations in the body's protective antioxidant systems in runners. For instance, vitamin C levels were higher with increased levels of training. However, increased training increases the requirement for antioxidant nutrients in order to prevent oxidative damage. A diet which is low or deficient in antioxidants could thereby increase the susceptibility of an athlete to illness.

Thus, while high training mileage appears to increase the risk of URTIs, other important mitigating factors may exist. Possibly, dietary supplements of antioxidants in athletes with increased needs and/or inadequate intakes could help prevent illness during intense training.

Vitamin C Supplementation in Ultra-marathon Runners

Peters et al. (1983, 1990) reported that individuals who competed in ultramarathons (races over 42 km) were at increased risk of URTIs during the post-race period compared to sedentary subjects. They found an increased incidence of URTIs of over fifty percent in the runners compared to age-matched sedentary subjects. Other researchers, as previously discussed, have also found an increased incidence of URTIs among long-endurance athletes (Heath et al. 1991, Nieman et al. 1989, Nieman et al. 1990).

Recently, Peters et al. (1993) suggested that the ultramarathoners, due to their extremely high relative incidence of URTIs in the post-race period, provide a good model to study the effects of vitamin C on resistance to infection.

A double-blind, placebo-controlled study was conducted to assess the effect of vitamin C supplements on ultramarathon runners and sedentary subjects (Peters et al. 1993). Ninety-two ultramarathon runners and age-matched sedentary subjects were randomly placed into placebo and treatment groups. Vitamin C (600 mg) or placebo was taken daily for 21 days prior to the 1990 90 km Comrades Marathon in South Africa.

Questionnaires were used to ascertain running history, the amount and intensity of usual training, dietary intake, and state of health during the 3 weeks before the race. In addition, a 24 hour dietary record was collected from each subject between days 10 and 14 before the race. Information regarding usage and doses of any dietary supplements was also collected. Two weeks following the race each subject was interviewed by telephone to determine race performance, compliance with supplement intake, and incidence of URTIs during the two weeks following the ultramarathon.

Sixty-eight percent of the runners who took placebos reported symptoms of URTIs during the two weeks following the ultramarathon, compared to thirty-three percent of the runners who took vitamin C. The incidence rates for URTIs among the sedentary subjects were extraordinarily high (45% in placebo-treated sedentary subjects and 53% in vitamin C-treated sedentary subjects) which contrasts with previous findings by Peters et al. (1983, 1990) and no explanations are offered.

Possibly, the runners infected the sedentary subjects resulting in high rates of infection in all groups. The mean duration and severity of URTIs symptoms was similar between the vitamin C and placebo-treated runners. In contrast, the duration and severity of symptoms of URTIs among the sedentary vitamin C group was less than for the placebo sedentary group.

Of interest, runners with the highest reported ratio of total training distance to training speed had the highest incidence of URTIs during the prerace training period, whereas race time was not related to risk of URTIs. Based on this finding, the authors stated that the cumulative stress of training may have predisposed the runners to infection during the prerace period.

Total intake of vitamin C averaged 1139 mg for the runners who took vitamin C, 494 mg for the runners who took placebos, 783 mg for the nonrunners who took vitamin C, and 280 mg for the nonrunners who took placebos. Mean supplementary vitamin C intake (apart from study supplementation) of vitamin C was 227 mg for the runners who took vitamin C, 209 mg for the runners who took placebos, 35.3 mg for the nonrunners on vitamin C, and 38.5 mg for the nonrunners on placebos. All the subjects had intakes from diet alone of vitamin C well above the Recommended Daily Allowance (RDA) of 60 mg. Notably, the dose of vitamin C used to reduce the incidence of URTIs in the ultramarathoners was quite high given the already relatively high total intake of vitamin C in the runners in the placebo group (about eight times the RDA).

While this study appears to have been well-designed, double-blind and placebo-controlled, a few limitations are worth mentioning. First of all, the subjects were all volunteers. Each runner had to select an aged-matched sedentary control for him or herself. This potentially introduces subject bias into the study due to using volunteers and due to the selection of friends and relatives as controls. However, this should not have affected the results of the vitamin C supplement on the runners.

The vast majority of the subjects were male: 82 male and 2 female runners completed the study. Hence, these results only apply to male ultramarathon runners and not to females.

The data involved subjective self-report data which relies on subject memory, honesty, and compliance with the study. However, the double-blind placebo-controlled design helped control for some of this potential bias.

Due to the collection of data on symptoms of URTIs, the researchers excluded any subjects with allergic rhinitis. Also, some of the sedentary subjects were excluded due to their not being entirely

sedentary. Unless they were exercising rigorously, it is doubtful that this would have affected their risk of URTIs.

It would be interesting to know whether vitamin C supplementation reduces the risk of URTIs in athletes who engage in less prolonged events (such as marathon runners). From this study, we do not know at what point an athlete might benefit from vitamin C supplementation.

Summary of Research Findings

The studies on runners and URTIs demonstrate that high annual running mileage (above 784 km or 486 miles) or training mileage (over 97 km or 60 miles per week) resulted in an increased risk of URTIs (Heath et al. 1991, Nieman et al. 1990). No differences were found in respiratory infections between 5 and 10 km runners and half marathoners (Nieman et al. 1989). It is possible that most of these runners had relatively low annual mileage (under 784 km or 486 miles per year, 14.5 km or 9 miles per week). Presumably, marathon runners would log greater distances than this per year.

Marathon runners who trained over 97 km per week (60 miles) had double the risk of URTIs compared to lower mileage runners (Nieman et al. 1990, Nieman 1992). Although marathon runners may not train all year, some runners log high mileage during the two-month period prior to a marathon (marathon training schedules often suggest peaking at 81 km or 50 or more miles per week). Thus the long-endurance athlete or marathon runner appears to be at an increased risk of URTIs compared to shorter distance runners. Of interest, Nieman et al. (1990) found that runners with fewer years of experience reported a higher incidence of URTIs, which suggests that there may be some type of adaptation to training and its effect on URTIs.

These data support the hypothesis by Nieman (1989, 1990) of a U-shaped curve where sedentary and extremely active people have a higher risk of URTIs than moderately active people. The increased resistance to URTIs among those who participate in regular moderate exercise could be of particular significance to those with compromised immune systems such as the elderly (Nieman et al. 1993).

Interestingly, Nieman et al. (1989b) and Berk et al. (1989) demonstrated hormonal and immune system alterations in marathon runners which lasted for up to 21 hours after exercise. These data suggest the possibility of a short period following exercise during which a subject is highly vulnerable to infection. If this is the case, precautions such as spacing out workouts and competitions and avoiding sick individuals during this time period may be protective. In the study by Robertson et al. (1991), alterations in the body's protective antioxidant systems were demonstrated in runners. For instance, vitamin C levels were higher with increased levels of training. However, increased training may require an increased intake of the antioxidant nutrients to prevent oxidative damage. A poor diet could thereby increase the susceptibility of the athlete to illness.

Given the recent findings by Peters et al. (1993) that vitamin C supplementation reduced by half the rate of URTIs among ultramarathon runners, it would be interesting to determine whether vitamin C might

have a beneficial effect on runners who engage in somewhat less prolonged activity.

In summary, while running mileage appears to play a role in the incidence of URTIs, important mitigating factors such as vitamin C supplementation may exist. Possibly, dietary supplements of antioxidants or forms of stress management could be used during intense training to help prevent illness in athletes.

Chapter Three Methodology

Study Population

The participants were recruited from all registered participants in the 1994 Duke City Marathon (DCM) and were found in part through previous (1992 and 1993) registration lists and finisher lists published in the Albuquerque Tribune. Previous marathon finishers were contacted and asked to participate in the study. In addition, subjects were recruited from local road races prior to the marathon to increase the number of subjects enrolled in the study two months prior to the race. Flyers were left in various sports stores and popular running locations in Albuquerque, and advertisements were placed in local newspapers.

An age and gender-matched sedentary group were obtained by including friends and coworkers of the marathon runners whenever possible. Additional sedentary subjects were recruited by flyers and word-of-mouth. Sedentary subjects completed an exercise questionnaire at baseline to confirm that they did not regularly participate in any aerobic exercise, including walking, cycling, jogging, etc.

The study was approved by the University of New Mexico Human Subjects Review Committee. An informed consent was obtained from all subjects prior to enrollment in the study.

Sample Size

The total number of marathon participants and ultimately, finishers, in the 1994 DCM restricted the number of study subjects. In 1992, 376 men and 90 women (466 total) completed the marathon and in 1993, 324 men and 80 women completed the marathon (404 total). In order to obtain an adequate sample size, potential marathon runners were contacted several months prior to the marathon and asked to participate in the study.

Given the greater interest in exercise, nutrition, and health among runners compared to the general population and the minimal time and effort required by the study for the majority of the study subjects, at least half of the runners were expected to consent to the study, or about 200 marathoners. Of these, a 10-20% rate of attrition was anticipated due to illnesses, injuries, and personal factors. Thus the final expected number of subjects was 160-180 marathoners. However, not all of these marathoners would have registered or have decided to run by the time the study was scheduled to begin. Thus, an estimated 100 marathon runners were expected to be enrolled in the study by two months before the race. Given the high incidence of URTIs among marathon runners, estimated at 43% during the two month period prior to the race (Nieman et al. 1990), a sample size of 110 runners would have been adequate to detect a 50% difference (as seen by Peters et al. 1993 in ultramarathoners) in URTIs between vitamin C and placebo treated runners with 80% power with an alpha of .05.

As it turned out, only 325 runners (256 males and 69 females) finished the full marathon in 1994, which was a substantially lower turn-out than in the previous two years.

Randomization and Dropouts

Prior to the start of the study, 104 potential marathon runners and 87 sedentary subjects had agreed to participate. The subjects were randomly assigned into either the vitamin C or the placebo treatment (n = 52 in each treatment group for the runners, n = 42 in the vitamin C treatment and n = 45 in the placebo treatment in the sedentary group). However, only 75 runners and 62 sedentary subjects actually began the study and filled out the questionnaires. Most of the runners who dropped out reported that they were not sure that they would run the full marathon either due to time constraints or to injuries.

At the start of the study, 137 subjects (75 marathoners and 62 sedentary subjects) were enrolled. Among the 75 marathon runners who actually began the study, 43 were in the vitamin C group and 32 were in the placebo group. Among the 62 sedentary subjects who began the study, 30 subjects were in the vitamin C group and 32 were in the placebo group. Out of the 137 subjects who were enrolled at the outset, 92 subjects completed the study.

Of the 45 subjects who began the study but did not complete it, 31 were marathon runners (13 vitamin C-treated and 18 placebo-treated) and 14 were sedentary controls (7 vitamin C-treated and 7 placebo-treated).

The reasons given by the marathon runners for not finishing the study included the following: injuries (1 placebo-treated), moved (2 vitamin C-treated and 1 placebo-treated), vitamin C side-effects (1 placebo-treated), too busy to train (2 vitamin C-treated, 5 placebo-treated), and the remaining 17 were lost to follow-up (7 vitamin-C treated, 10 placebo-treated). Among the sedentary subjects, the reasons for not completing the study included: vitamin C side-effects (1 vitamin C-treated and 1 placebo-treated), did not want to take the supplements (2 vitamin C-treated and 2 placebo-treated), too busy to participate (3 vitamin C-treated, 2 placebo-treated), medical problems (1 placebo-treated), had trouble remembering to take the supplements (1 vitamin C-treated), and became athletic (1 placebo-treated).

Study Subjects Dropped from Analysis

Two sedentary subjects who were receiving vitamin C supplements were omitted from the analysis due to not taking the supplements as determined from a pill count performed at the end of the study. These two subjects took under 60% of the study supplements. The 60 % criterion was determined post-hoc based on the fact that the remainder of the subjects appeared to have taken most of the supplements whereas these two individuals were extreme outliers (see Study Compliance in Chapter Four). These two subjects were included in the discussion of dropouts above (one reported that he had difficulty remembering to take the supplements and the other complained of side-effects due to the supplements).

Thus the final sample size was 92 subjects (44 runners and 48 sedentary subjects). Of these, 14 runners were in the placebo group

and 30 were in the vitamin C group and 25 sedentary subjects were in the placebo group and 23 were in the vitamin C group.

Time-line: Baseline, Pre-marathon, Post-marathon

Questionnaires and vitamin C or placebo tablets were distributed to the study subjects at baseline which was 2 months prior to the marathon and prior to any study intervention. Subjects were restricted to a maximum of 200 mg per day of self-supplementation with vitamin C.

However, supplementation prior to baseline was not controlled. Subjects who volunteered for blood drawings had the initial blood drawn at baseline in the University of New Mexico Clinical Research Center (CRC).

After two months of vitamin C or placebo supplementation, study subjects who had agreed to have blood drawn were again seen in the CRC.

This occurred one week prior to the marathon (pre-race). In addition, a sub-sample of runners had blood drawn in the CRC two days following the marathon (post-race).

Study Design

Runners and sedentary subjects were randomly assigned to either the vitamin C or the placebo group for the two month period prior to and one month following the 1994 DCM. While the study subjects actually received the supplements for a total of 92 days, for analytical purposes, only days 7 through 90 of the intervention were counted in the logistic regression (weeks 1 through 8 were before the marathon and weeks 9 through 12 were after the marathon). However, the entire 3 months (92 days) of the study was used in the determination of duration and severity of colds. Finally, any colds which occurred on marathon day were considered to occur after the race.

The study was conducted as a double-blind, placebo controlled trial. Each subject was assigned a number upon entry into the study and was randomly assigned into treatment or placebo group by a computer generated randomization of the assigned numbers. Separate randomization lists were maintained for runners and for sedentary subjects.

Vitamin C (500 mg) and placebo supplements were obtained at no charge from Hoffman-La Roche (Nutley, NJ). Subjects were instructed to take two tablets each morning with breakfast, specifically at 8 a.m. on the mornings of the day immediately prior to blood draws. Each subject was given a training log for the two month period prior to and one month period following the race. Subjects were instructed to log their training distances and running times daily. Subjects were also instructed to complete a respiratory symptom report sheet on each day that they had a runny nose, cough, or sore throat.

Data Collection and Questionnaires

Consent forms, questionnaires, and vitamin C and placebo supplements were either given to the subjects in person at the Clinical Research Center or sent out in a single mailing prior to the start of the study.

Stress levels were determined for all subjects at baseline (two months prior to the marathon) using three scales obtained from Cohen et al. (1991) including: 1) a 10-item perceived stress scale, 2) a list of recent experiences, adapted from Henderson et al. (1981), and 3) a negative-affect (mood) scale which uses 15 items adapted from Zevon and Tellegen (1981).

The stress level questionnaire was readministered to subjects at the blood draw one week before the marathon (n = 33 runners, 35 sedentary subjects). Each of the three stress scales were considered separately and also as a composite by summing each of the stress scores (using the "quartiles" for stress levels per Cohen et al. 1991).

Symptoms and duration of URTIs was assessed throughout the study period using a semi-quantitative list of respiratory symptoms (see Appendix). Subjects were asked to fill out the respiratory symptoms report sheet on each day that they had a runny nose, cough, or sore throat. Subjects were asked to provide the dates for any colds in other household members throughout the study. In addition, subjects were encouraged to report to the nurse practitioner at Urgent Care at the University Hospital for diagnosis of URTI when they suspected that they had a cold to provide clinical evidence of URTI. Only one subject pursued this option; therefore this part of the study was not included in the analysis.

Usual dietary intake was determined on 135 subjects upon entry into the study (at baseline and before beginning the intervention) using the Health Habits and History Questionnaire: Diet History and Other Risk Factors Dietary Analysis System (HHHQ-DIETSYS Analysis Software, Version 3.0, National Cancer Institute, 1993) (see Appendix).

Scannable mark-sense dietary questionnaires were purchased from National Computer Systems, Inc., Minneapolis MN) and scanned by Survey & Ballot Systems, Inc. (Eden Prairie, MN). The computer software for analyzing the scanned responses was available from the National Cancer Institute.

The questionnaire was modified to include foods commonly eaten in the Southwest that are high in vitamin C (Pareo-Tubbeh et al. 1994, Pareo-Tubbeh et al. 1995, Koehler et al. 1995). The Block Food Frequency Questionnaire was chosen as it reflects usual intake and can be used to rank individuals or groups by nutrient intake. In addition, the questionnaire can be self-administered and the burden on both the subject and the researcher is lower than with diet records. Any supplement use was also recorded. Usual intake of calories, percent of calories from carbohydrates, fat, and protein, cholesterol intake, and intake of major vitamins and minerals was determined. Vitamin C intake from diet plus supplements was determined for each subject. No active

interventions were made during this study, but a registered dietitian was available to answer any questions asked by the subjects regarding nutrient intake. Although 135 subjects completed the dietary questionnaire, six individuals were dropped from the analysis due to questionable accuracy of questionnaires.

Two subjects did not complete the food frequency questionnaire. The HHHQ-DIETSYS provides for edit checking of data; six subjects were excluded from the dietary analysis due to questionable accuracy of their responses. Of these, 5 subjects were dropped for reporting too few food eaten (4 males reported eating less than 4 food items per day and one female reported eating less than 3 food items per day) and one subject was dropped for coding all food items as the same (medium) serving size.

Monthly running logs were developed based on similar logs used by Nieman et al. (1990) to determine training mileage and intensity.

Additional descriptive data, such as prior running history, marathon experience, and exposure to children at home and at work, were collected by questionnaire (see Appendix). Subjects were also asked whether they thought they were on vitamin C or placebo at the end of the study.

TABLE 5. Data Collection: Time-line for Distribution of Study
Questionnaires and Determination of Biomedical Parameters

Baseline (Prior to Study Intervention)

Consent Forms, Questionnaires (Stress, Dietary, Demographic)
administered to all subjects (n = 92; 30 vitamin C- and 14
placebo-treated runners, 23 vitamin C- and 25 placebo-treated
sedentary subjects).

Plasma Vitamin C Levels
determined on sub-sample of subjects (n = 69; 25 vitamin C- and 8
placebo-treated runners, 19 vitamin C- and 17 placebo-treated
sedentary subjects).

Lymphocyte Proliferation
determined on 16 marathon runners (8 vitamin C- and 8 placebo-
treated).

One Week Pre-Marathon (After Two Months of Intervention)

Stress Questionnaires
re-administered to sub-sample of subjects (n = 69; n = 25
vitamin C- and 8 placebo-treated runners, 19 vitamin C- and 17
placebo-treated sedentary subjects).

Plasma Vitamin C Levels
determined on sub-sample of runners and sedentary subjects (n
= 69).

Lymphocyte Proliferation
determined on 16 runners.

Two Days Post-Marathon

Plasma Vitamin C Levels
determined on marathoners only in sub-sample (n = 33; 25 vitamin
C and 8 placebo-treated).

Independent Variables

The following independent variables were determined from questionnaires and training logs: weekly running mileage, training intensity, and stress level. In addition, the following descriptive variables were examined.

- gender
- age
- marathon race time
- number of marathons previously completed
- number of colds in household during the study
- occupation and exposure to children at work
- number of people and children in household
- usual vitamin C intake and intake of other nutrients
- supplement use

Dependent Variables

The overall hypothesis of this study was that vitamin C supplementation influences the risk of URTIs among marathon runners. Therefore, the dependant (outcome) variables included incidence and duration of upper respiratory tract infection (URTIs) as determined by self-report. Incidence (total number of cases) and the percentage of subjects in each treatment group with at least one URTI during the two months prior and one month following the marathon were determined.

Duration of each URTI was determined based on self-report in the respiratory symptom logs. The severity score was determined by summing up the severity of any symptoms present for each day of a cold on a scale of 0 (not present), 1 (mild), 2 (moderate), and 3 (severe). Possible symptoms included: cough, nasal discharge, sneezing, stuffy nose, sore throat, headache, malaise (tired out), chilliness, shaking chills, fever, hoarseness, aching muscles or joints, watery or burning eyes.

In addition, biomedical parameters including plasma vitamin C and lymphocyte proliferation were assessed on a subset of the study sample who volunteered to give blood samples. Blood samples were drawn in the CRC three times during the study: presupplement (blood was drawn one to two weeks prior to supplementation), the week before the race, and post-race (runners only). Plasma vitamin C levels were determined on all blood samples by an automated procedure using 2,6 dichloroindophenol (Garry et al. 1973, Garry et al. 1982) in the Clinical Nutrition Laboratory at the University of New Mexico School of Medicine.

Spectra Cell Inc. (Houston, TX) has developed a functional nutrient analysis system based on lymphocytes which are exposed to the mitogen PHA and incubated for four days. Tritiated thymidine is then added and the lymphocytes are cultured for one more day (Shive 1984,

Shive et al. 1986, Bucci 1993). The proliferative rate can then be assessed by measuring reactivity with and without adding various nutrients to the media to detect "deficiency". Although this method is somewhat new, the measure of lymphocyte proliferation (^3H , cpm $\times 10^{-3}$) is of interest in this study. However, it is noteworthy that Anderson et al. (1980) reported stimulation of lymphocytes with daily ingestion of 1, 2, and 3 gm of vitamin C in five healthy adults with much higher doses of mitogens than Spectra Cell Laboratories (25 and 50 ug/ml compared to 2 mg/L or 2 ug/ml).

In addition, Spectra Cell also measures antioxidant status by adding cumene hydroperoxide as an oxidative stress to the assay and then determining lymphocyte proliferation by assessing tritiated thymidine after five days incubation. Antioxidant status is thus determined as the percentage growth compared to growth in media without cumene hydroperoxide.

Spectra Cell, Inc. agreed to determine lymphocyte proliferation and antioxidant status for 10 vitamin C- treated runners and 10 placebo-treated runners at baseline and pre-race. Blood specimens were sent to Spectra Cell by overnight mail at baseline (prior to supplement administration) and during the week before the marathon.

Data Analysis and Results

The main hypothesis of this study was:

1. vitamin C supplementation influences the risk of URTIs among marathon runners. Therefore, the dependent variables included incidence and duration of URTIs as determined by the respiratory symptoms report sheets. The Statistical Analysis System (SAS Institute, Cary, NC) was used to perform a logistic regression of URTI incidence. Variables examined included a treatment factor (vitamin C or placebo) and a group factor (runner or sedentary). In addition, analysis of variance (ANOVA) was performed for both URTI duration and for symptom scores with a treatment factor (vitamin C or placebo) and a group factor (runner or non-runner). Since ANOVA assumes a normal distribution, transformation of data was considered. Since the data revealed unequal variances, log transformation was performed.

Additional study hypotheses were:

2. Vitamin C supplementation affects plasma vitamin C levels in marathon runners and in sedentary subjects over time.
3. Vitamin C supplementation has no effect on lymphocyte proliferation in marathon runners.

The effect of vitamin C on plasma levels (hypothesis 2) was examined using a mixed design repeated-measures ANOVA on two time points, with a treatment factor (vitamin C or placebo) and a group factor (runner or non-runner). A second repeated-measures ANOVA was performed on runners only at three time points, with a treatment factor (vitamin C or placebo). Repeated-measures ANOVA allowed for testing differences between groups and times as well as for interactions.

The effect of vitamin C on lymphocyte proliferation in marathon runners (hypothesis 3) was examined using repeated-measures ANOVA with two time points, with a treatment factor (vitamin C or placebo).

Study sub-hypotheses included the following.

1. High stress level is predictive of URTIs in marathon runners.
2. High training mileage is predictive of URTIs in marathon runners.
3. Training intensity (pace) is predictive of risk of URTIs in marathon runners.

Multiple logistic regression was used to determine whether stress level was predictive of URTIs in marathon runners (sub-hypothesis 1) since the outcome variable (URTIs) was a binary variable. Logistic regression was also used to examine the association between training mileage and URTIs (sub-hypothesis 2) and the association between training intensity (pace) and URTIs (sub-hypothesis 3). Stepwise logistic regression was used to obtain the best models prior to running the final multiple logistic regression analyses.

In addition, descriptive data, such as gender and age were examined and if differences were found, these variables were used as covariates in the above analyses.

Chapter Four Results

Subject Characteristics

Ninety-two subjects (44 marathon runners and 48 sedentary subjects) completed the entire study and were retained in the analysis (see Chapter 3 for detailed information on randomization and dropouts).

Twenty-five placebo-treated sedentary subjects, 23 vitamin C-treated sedentary subjects, 14 placebo-treated runners, and 30 vitamin C-treated runners completed the study. Since fewer placebo-treated runners completed the study compared to vitamin C-treated runners, an analysis of the number of dropouts (after giving informed consent) was performed and revealed no significant treatment or running group differences in number of dropouts (Fisher's Exact test, $p = 0.08$). In addition, an analysis of the dropout rates was also performed on the subjects prior to enrollment (before giving informed consent) and also showed no significant treatment or running group differences in dropout rate (Fisher's Exact Test, $p = 0.12$).

As shown in Table 6, eleven of the marathon runners (25%) and seventeen of the sedentary subjects (35.4%) were female. The average age of the subjects was 42 years for runners and 44 years for sedentary subjects. Average height and weight was 1.74 meters and 69.8 kg for marathoners and 1.75 meters and 80.3 kg for sedentary subjects. Percentage body fat at baseline per skinfolds (Durnham and Womersley 1974) was higher in sedentary subjects (30.1%) compared to runners (24.8%). Thus, the marathoners and sedentary subjects were similar in respect to age and height, whereas the sedentary subjects tended to weigh more and have a higher percentage of body fat (Kruskal-Wallis nonparametric test, $p = 0.0101$). Percent body fat was lower (16.1%) among the runners when determined one week prior to the marathon using a different set of equations thought to be more accurate for an athletic population (Jackson and Pollock 1978, Jackson et al. 1980) compared to baseline values.

According to running histories obtained prior to the study, the marathoners had been running for 13.4 years, ran an average of 47.4 km per week (29.4 miles per week), had a marathon personal best of 3 hours 23 minutes and had completed 16.9 marathons.

TABLE 8. Upper Respiratory Tract Infections (URTIs) (mean \pm SEM): Number of Subjects and Percentage of Subjects with URTIs, Total Number of Cases of Reported URTIs, Mean Duration and Severity of URTIs for Vitamin C and Placebo-Treated Marathon Runners and Sedentary Controls.

	n	# and % of subjects with URTIs	Total # of cases of (days) ^{**}	Mean Duration of URTIs ^{***}	Mean Severity of Symptoms
Runners Vitamin C	30	10/33.3	15	5.4 \pm .9	42.6 \pm 7.4
Runners Placebo	14	6/42.9	12	2.7 \pm .6	17.8 \pm 7.5
Sedentary Vitamin C	23	10/43.5	14	2.5 \pm .3	16.1 \pm 3.9
Sedentary Placebo	25	8/32.0	12	4.2 \pm 1.0	37.4 \pm 15.2

* Treatment-by-running group effect, p = 0.02 (ANOVA).

** Treatment-by running group effect, p = 0.02 (ANOVA).

+ Treatment difference among runners, p = 0.01 (t-test).

** Treatment difference among runners, p = 0.01 (t-test).

TABLE 6. Subject Characteristics (mean \pm SEM and selected ranges) at Baseline (prior to Vitamin C Supplementation).

	Marathon Runners (n = 44)		Sedentary Subjects (n = 48)	
	Mean	Range	Mean	Range
Females (n, %)	11 (25%)		17 (35.4%)	
Placebo-treated (n, %)	14 (31.8%)		25 (52.1%)	
Vitamin C-treated (n)	30		23	
Age (yr)	42 \pm 0.81	24-64	44 \pm 0.71	22-65
Height (m)	1.74 \pm 0.01		1.75 \pm 0.01	
males	1.77 \pm 0.01	1.65-1.96	1.80 \pm 0.01	1.68-1.98
females	1.66 \pm 0.02	1.57-1.75	1.67 \pm 0.01	1.57-1.75
Weight (kg)	69.8 \pm 0.97*		80.3 \pm 1.71*	
males	73.6 \pm 1.75	55.3-97.5	87.0 \pm 3.74	59.0-130.2
females	57.9 \pm 1.17	51.3-63.5	67.7 \pm 3.33	49.9-94.3
Body Fat % #	24.8 \pm 0.78**		30.1 \pm 0.74**	
males	22.8 \pm 1.46	8.6-35.6	27.3 \pm 1.41 ³¹	14-38
females	32.4 \pm 1.15	29.0-38.0	35.8 \pm 1.90	25-47
Body Fat % +	16.1 \pm 0.67		-	
males	14.3 \pm 1.22	4.6-22.0	-	
females	22.6 \pm 1.71	17.9-30.5	-	
Km Run (per wk)	47.4 \pm 2.94	13-113	-	
Years Run	13.4 \pm 0.70	3-32	-	
Marathon Best (min)**	205.5 \pm 4.94	137-299	-	
Marathons Completed	16.9 \pm 2.94	0-140	-	

* p = 0.03

** p = 0.01

per skinfolds at baseline (Durnham and Womersley 1974), n = 33 runners (26 males and 7 females and 39 sedentary subjects (26 males and 13 females).

+ per skinfolds at follow-up after two months of vitamin C supplementation (Jackson and Pollock 1978, Jackson et al. 1980), n = 33 runners.

** n = 36 subjects who previously completed marathons.

Study Compliance

Compliance with the study was determined by questionnaire and by verbal report at follow-up appointments at the Clinical Research Center among a subset of the study participants (n = 69; 35 marathon runners and 34 sedentary subjects). Reported percentage of the time that the subjects took the study supplements was $100\% \pm 0.7$ and $99\% \pm 0.6$ (mean \pm SEM) among the placebo and vitamin C-treated runners, respectively, and $95\% \pm 1.4$ and $93\% \pm 3.9$ (mean \pm SEM) among the placebo and vitamin C-treated sedentary subjects, respectively. This self-reported compliance was significantly higher among the runners than among the sedentary subjects (Kruskal-Wallis nonparametric test, $p = 0.004$).

In addition, a pill count was performed for each subject (n = 94). The pill count revealed that two subjects, both sedentary subjects on vitamin C supplements, had taken less than 60 percent of the study supplements. This finding was vastly different from the remainder of the study subjects and a post-hoc decision was made to drop them from the analysis (final sample size = 92).

No significant differences between treatment groups were found in the number of tablets left at the end of the study beyond what they would have had left if fully compliant (Kruskal-Wallis nonparametric test, $p = 0.13$). Placebo and vitamin C-treated marathon runners had 9.9 ± 4.4 and 4.0 ± 1.0 (mean \pm SEM) supplements left (beyond extras), respectively, at the end of the study while placebo and vitamin C-treated sedentary subjects had 7.7 ± 2.3 and 14.9 ± 3.9 (mean \pm SEM) tablets left, respectively.

Subjects were also queried regarding which treatment they believed that they were taking during follow-up appointments at the Clinical Research Center or by questionnaire. McNamara's test was used to determine whether the subjects knew which treatment they were actually on. Subjects without any idea which treatment they were on were excluded from this analysis.

In the sedentary group, no significant differences were found between actual and believed treatment (McNamara's test, $p = 0.51$ for sedentary group). In contrast, more of the marathon runners tended to be wrong when asked which treatment they believed that they were on ($p = 0.07$ for runners). In particular, more runners on vitamin C believed that they were on placebo. When the runners and sedentary subjects were combined, there was a significant difference in the actual versus believed treatments with more subjects being incorrect in guessing their treatment ($p = 0.04$ for runners and sedentary group combined). Again, more subjects on vitamin C were incorrect in guessing their treatment than the subjects on the placebo treatment.

TABLE 7. Believed Treatment during Vitamin C or Placebo Supplementation in a Subset of Marathon Runners and Sedentary Controls (n = 73).

	Believed Treatment		
	Placebo	Vitamin C	No Idea
Sedentary Placebo	9	3	6
Sedentary Vitamin C	6	6	7
Runners Placebo	4	2	4
Runners Vitamin C	9	6	11

 McNamara's test (excluding "No Ideas") p = 0.51 for sedentary subjects, p = 0.07 for runners, p = 0.04 for sedentary subjects and runners combined.

Hypothesis 1: Vitamin C Supplementation Decreases the Incidence of Upper Respiratory Tract Infections (URTIs) among Marathon Runners

The main hypothesis of this study (hypothesis 1) was that vitamin C supplementation decreases the incidence of URTIs among marathon runners. The incidence of reported URTIs for vitamin C and placebo-treated runners and vitamin C and placebo-treated sedentary subjects during the study period (two months prior to and one month following the 1994 Duke City Marathon) is shown below in Table 8.

Fifty-three cases of upper respiratory tract infections (URTIs) were reported during the study, 26 among the sedentary subjects and 27 among the marathon runners. Of the 53 reported cases of URTIs during the study period, 31 were among vitamin C-treated subjects (15 runners and 14 sedentary subjects) and 25 were among placebo-treated subjects (12 runners and 12 sedentary subjects). Fisher's Exact Test was performed on the marathon runners and on the sedentary subjects to determine whether there were any treatment differences in the number of URTIs. This analysis included both subjects with and without colds, unlike the ANOVA discussed below. Fisher's Exact Test revealed no differences in reported cold incidence between the four treatment groups (p = 0.79). When treatment alone was examined by combining the runners and sedentary subjects, no differences were found in reported URTIs (p = 1.0).

TABLE 8. Upper Respiratory Tract Infections (URTIs) (mean \pm SEM): Number of Subjects and Percentage of Subjects with URTIs, Total Number of Cases of Reported URTIs, Mean Duration and Severity of URTIs for Vitamin C and Placebo-Treated Marathon Runners and Sedentary Controls.

	n	# and % of subjects with URTIs	Total # of cases of (days) ^{**}	Mean Duration of URTIs ^{***}	Mean Severity of Symptoms
Runners Vitamin C	30	10/33.3	15	5.4 \pm .9	42.6 \pm 7.4
Runners Placebo	14	6/42.9	12	2.7 \pm .6	17.8 \pm 7.5
Sedentary Vitamin C	23	10/43.5	14	2.5 \pm .3	16.1 \pm 3.9
Sedentary Placebo	25	8/32.0	12	4.2 \pm 1.0	37.4 \pm 15.2

* Treatment-by-running group effect, p = 0.02 (ANOVA).

** Treatment-by running group effect, p = 0.02 (ANOVA).

+ Treatment difference among runners, p = 0.01 (t-test).

** Treatment difference among runners, p = 0.01 (t-test).

In addition, odds ratios were calculated for the risk of acquiring an URTI during the study. The odds ratio for acquiring a cold among the vitamin C-treated runners was 0.67 (95% confidence interval = 0.18 - 2.45), whereas it was 1.64 (95% confidence interval = 0.50 - 5.3) among the vitamin-C treated sedentary subjects. However these two odds ratios were not significantly different from 1.0 (Mantel-Haenszel Inference, $p = 0.54$ for runners and $p = 0.41$ for sedentary subjects), nor did they differ from each other (Zelen's Exact Test, $p = 0.39$). Thus, the common odds ratio for acquiring an URTI was calculated to be 1.1 (Mantel-Haenszel Inference; $p = 0.84$ differs from 1.0, 95% confidence interval = 0.46 - 2.61). Thus, no significant differences were found in the risk of URTIs between vitamin C and placebo-treated runners or between vitamin C and placebo-treated sedentary subjects.

Duration and Severity of Cold Symptoms

ANOVA was used to determine whether there were any treatment or running group differences in either the number of respiratory symptoms (severity score) or the duration of URTI symptoms. In the analyses of duration and severity of cold symptoms, the unit of analysis was each case of reported URTI, rather than each subject. Log values were used for both the number of symptoms and for the duration of symptoms due to unequal variance in the untransformed numbers (the original data are shown in Table 8 for ease in interpretation).

The ANOVA of the duration of cold symptoms revealed no differences between running groups (marathoners versus sedentary subjects) ($p = 0.46$) or treatment groups ($p = 0.41$). However, a significant treatment-by-running group effect was found ($p = 0.02$). Among the marathon runners, a significant treatment difference was found for the duration of cold symptoms (t-test, $p = 0.01$), with the vitamin C-treated subjects having a longer duration of cold symptoms (mean \pm SEM = 5.4 ± 0.9 days versus 2.7 ± 0.6 days; untransformed values reported for ease of interpretation). By contrast, no treatment difference was found among the sedentary subjects for duration (t-test, $p = 0.32$) (mean \pm SEM = 2.5 ± 0.3 versus 4.2 ± 1.0 days, untransformed scores for vitamin C and placebo-treated sedentary subjects).

ANOVA of the number of symptoms (severity score, see Table 8) revealed no running group effect ($p = 0.83$) or treatment difference ($p = 0.43$), but did find a running group-by-treatment effect ($p = 0.02$). A significant treatment difference was found for the severity score for runners (t-test, $p = 0.01$), with the vitamin C-treated runners reporting more symptoms (severity score mean \pm SEM = 42.6 ± 7.45 versus 17.8 ± 7.47 , untransformed values).

Among the sedentary subjects, no treatment difference was found for the severity score (t-test, $p = 0.23$) (mean \pm SEM = 16.1 ± 3.94 versus 37.4 ± 15.24 , untransformed scores, for vitamin C and placebo-treated sedentary subjects, respectively).

Post-Race Results

URTI incidence was examined for the two weeks immediately following the marathon (see Table 9). Seven marathon runners (15.9% of the runners) reported URTIs during these two weeks, whereas only four sedentary subjects (8.3% of the sedentary subjects) had cold symptoms during this time period (Fisher's Exact test, $p = 0.34$). When the marathoners and sedentary group were examined together 12.0% had cold symptoms during the two weeks following the race (95% confidence interval from 6.1 to 20.4%). However, no treatment differences were found in the number of reported URTIs during the two weeks following the marathon when the marathoners and sedentary group were examined together (Fisher's Exact test, $p = 0.19$). Interestingly, no URTIs were reported among the sedentary group during the week immediately following the marathon, whereas four marathon runners (three in the vitamin C group and one in placebo group) reported URTIs during this week (9.1%, 95% confidence interval from 2.0 to 18.9%).

TABLE 9. Upper Respiratory Tract Infections (URTIs): Number of URTIs Reported and Percentage of Subjects with URTIs during the Two Weeks Following the Marathon.

	n	# of URTIs Reported	Percentage with URTIs
Runners Vitamin C	30	3	10.0%
Runners Placebo	14	4	28.6%
Runners (Total)	44	7	15.9%
Sedentary Vitamin C	23	1	4.4%
Sedentary Placebo	25	3	12.0%
Sedentary (Total)	48	4	8.3 %

Covariables and URTI

The results of the multiple logistic regression are shown in Tables 10 and 11. Multiple logistic regression analysis was used to determine the relationship between incidence of reported URTI (the binary response variable) and ordinal explanatory variables such as age, gender, running mileage, stress level, alcohol intake, and plasma vitamin C concentrations. The three months of the study (time) were included in the model by creating two binary variables. The final regression models were run with and without accounting for time and the results were essentially identical. Regression models were run for all subjects together and for marathon runners and sedentary subjects separately.

Multiple logistic regression analysis of all subjects combined showed no running group or treatment differences. However, older subjects were at greater risk of URTIs compared to younger subjects (parameter estimate 0.0760, odds ratio 1.079 per year, p = 0.0002). Alcohol intake was inversely related to risk of URTIs; however the majority of the subjects (82.6%) reported drinking less than one drink per day, while 5.4% reported drinking one drink per day, 7.6% reported drinking 1 - 2 drinks per day, and 4.4% reported drinking more than 2 drinks per day. No running group or treatment differences were found for alcohol intake (Kruskal-Wallis nonparametric test, p = 0.44). Females tended to have more reported URTIs than men, but this finding was not significant when added to the model (parameter estimate 0.4002, odds ratio = 1.492, p = 0.27).

TABLE 10. Multiple Logistic Regression: Influence of various factors on Reported URTIs including Running Group, Treatment, Age (per year) and Alcohol Intake in Marathoners and Sedentary Subjects (adjusting for month).

	Parameter	Odds Estimate	Probability Ratio	Confidence > Chi-Square	Interval	Variable
						-----Running
Group	0.2908	1.338	p = 0.40	0.7,	2.7	
Treatment	0.4519	1.571	p = 0.21	0.8,	3.2	
Age (per yr)	0.0760	1.079	p = 0.0002	1.04,	1.1	
Alcohol	-0.3934	0.675	p = 0.01	0.5,	0.9	

Running variables such as average weekly running mileage and average pace could not be examined in this model without excluding sedentary subjects due to missing values; therefore a separate logistic regression analysis was performed on marathon runners only (see Table 11). In the analysis of marathon runners alone, age and alcohol intake

were no longer significant. Several running variables were related to risk of URTI. Specifically, the more marathons previously run, longer marathon personal bests, faster training paces, and shorter longest runs of the week were associated with an increased risk of URTIs. In addition, females were at greater risk of URTIs compared to males. However, marathon personal best was no longer significant when gender was added to the model, implying a relationship between these two factors. Therefore, marathon personal best was omitted from the model.

TABLE 11. Multiple Logistic Regression: Influence of Various Factors on Reported URTIs including Treatment, Gender, Number of Marathons Previously Run, Longest Run per Week (Maxmi), and Running Pace for Marathon Runners (adjusting for month).

Variable	Parameter Estimate	Odds Ratio	Probability > Chi-Square	Confidence Interval
Treatment	0.0757	1.079	P = 0.89	0.4, 3.1
Gender	1.1182	3.059	P = 0.05	1.0, 9.6
# of Marathons	0.0187	1.019	P = 0.03	1.0, 1.04
Maxmi	-0.1504	0.860	P = 0.03	0.8, 1.0
Pace	0.8308	2.295	P = 0.04	1.0, 5.1

Multiple logistic regression was also performed on sedentary subjects. As with the analysis of runners and the sedentary group combined, only age (parameter estimate 0.0833, odds ratio 1.087, $p = 0.01$) and alcohol intake (parameter estimate -0.5042, odds ratio 0.604, $p = 0.02$) were significant when adjusted for month and treatment. Although six sedentary subjects smoked, cigarette smoking was not a significant factor when included as a covariate in the logistic regression model.

Logistic regression was also used to determine the influence of each of the three-part stress scores or the composite stress index on reported cases of URTIs (see Study Sub-Hypothesis 1, below). None of the stress scores were significantly related to incidence of URTIs (p -values ranged from 0.36 to 0.75).

No treatment or running group differences were significant in any of the regression analyses. Furthermore, plasma vitamin C concentrations following two months of supplementation were not related to the incidence of URTIs using multiple logistic regression among the sub-sample ($n = 69$, $p = 0.22$ for one week pre-marathon and $p = 0.20$ for two days post-marathon for marathon runners, $p = 0.65$ for sedentary subjects).

Biomedical findings

Hypothesis 2: Vitamin C Supplementation Increases Plasma Vitamin C Levels

Plasma vitamin C concentrations are shown in Figure 1. Plasma vitamin C was measured at baseline, pre-marathon, and two days post-marathon (runners only) on a sub-sample of subjects who were willing to have blood drawn at the Clinical Research Center (see Chapter 3: Timeline for more detail on methodology). Among the runners, 33 subjects had blood drawn (25 in the vitamin C group and 8 in the placebo group).

As with the study as a whole, more runners in the vitamin C treatment group ($n = 30$) had blood drawn compared to runners in the placebo group

(14); however, this was believed to be due to chance since the analysis of dropouts revealed no significant difference between the number of dropouts between treatment or running groups (Fisher's Exact Test, $p = 0.16$) and also the study was double-blind with random assignment into treatment groups. In the sedentary group, 36 subjects had blood drawn at baseline and prior to the marathon (19 vitamin C-treated and 17 placebo-treated).

A three-way (treatment, time, and running group) repeated measures ANOVA was used to examine whether vitamin C concentrations differed between baseline and post-supplementation in the vitamin C and placebo-treated runners and sedentary subjects (Hypothesis 2). A significant effect was found for running category (marathoners versus sedentary subjects) ($p = 0.0001$) and for treatment-by-running category ($p = 0.03$), whereas no effect was found for treatment alone ($p = 0.10$). Thus, a treatment effect was found, but it differed between the runners and the sedentary subjects. As shown in Figure 1, the runners had higher plasma vitamin C concentrations at baseline compared to sedentary subjects. In the runners, vitamin C increased (not significant) in the vitamin C-treated group and decreased in the placebo-treated group (not significant). In the sedentary subjects, vitamin C concentrations increased in the vitamin C-treated subjects whereas no change was found in the placebo-treated subjects.

A two-way (treatment and time) repeated-measures analysis of variance was performed on runners only to determine if there were any significant differences in vitamin C over the three time points (baseline, one week pre-marathon, and two days post-marathon).

No treatment difference was found for vitamin C ($p = 0.46$); upon closer examination, baseline versus one week before the marathon (pre-marathon) vitamin C concentrations were not significantly different ($p = 0.12$) whereas pre-marathon vitamin C concentrations were significantly different from post-marathon (repeated measures ANOVA, $p = 0.04$).

In addition, an analysis of covariance was performed which adjusted for baseline plasma vitamin C concentrations. As expected from the results of the ANOVA above, a significant treatment effect was found for plasma vitamin C concentrations one week prior to the race ($p = 0.0001$) and two days post-marathon (runners only) ($p = 0.0002$). Thus by holding the baseline levels constant, the analysis of covariance in effect removed the running group-by-treatment interaction and demonstrated a significant treatment effect.

In addition, the relationship between usual dietary intake and total intake of vitamin C and baseline plasma vitamin C concentrations were examined in 92 subjects with available data (including dropouts from the study). Total vitamin C intake was not significantly related to baseline plasma vitamin C levels (regression analysis, $r^2 = 0.0183$, $p = 0.15$), whereas dietary intake of vitamin C was positively related to plasma vitamin C levels ($r^2 = 0.2505$, $p = 0.01$). In addition, a significant effect was found for running group ($p = 0.0001$ for both dietary and total vitamin C intake).

Hypothesis 3: Vitamin C Supplementation has no effect on Lymphocyte Proliferation in Marathon Runners

Lymphocyte proliferation was determined on 8 vitamin C-treated and 8 placebo-treated marathon runners at baseline and one week pre-marathon. Logs of lymphocyte proliferation were used in the analysis due to a large variation in the untransformed scores. No differences were found between the treatment groups at baseline (t-test, $p = 0.33$).

Paired t-tests were performed on the logs to determine if there were

FIGURE 1. Vitamin C concentrations (mg/dl) at baseline (prior to vitamin C supplementation), one week before the marathon (after two months of vitamin C supplementation), and two days post-marathon (for runners only) in vitamin C ($n = 25$) and placebo-treated ($n = 8$) runners and vitamin C ($n = 17$) and placebo-treated ($n = 19$) sedentary subjects (mean \pm SEM).

any changes between pre and post-supplementation within each treatment group ($p = 0.79$ for placebo group, $p = 0.08$ for vitamin C group). Curiously, the change in the vitamin C group was in the opposite direction as expected (see Table 12 below). One vitamin C-treated subject was considered a potential outlier; however, results remained similar when examined without this subject ($p = 0.06$). Similarly, no difference was found between the amount of change in the placebo versus the vitamin C groups (t-test, $p = 0.31$).

TABLE 12. Lymphocyte Proliferation (Untransformed Data and Logs) and Antioxidant Function at Baseline and Post-Supplementation in Vitamin C and Placebo-Treated Runners (mean \pm SEM).

	Vitamin C Runners	Placebo Runners
	n = 8	n = 8
Lymphocyte Proliferation (cpm ^3H)		
Untransformed Data:		
Baseline	8432 \pm 1810	5824 \pm 1209
Post-Supplementation	5430 \pm 1226	13009 \pm 8892
Logs:		
Baseline	3.8373 \pm 0.1133	3.6548 \pm 0.14269
Post-Supplementation	3.6277 \pm 0.1272	3.7207 \pm 0.18490

Antioxidant Function (% of control)		
Baseline	42.00 \pm 9.112	31.13 \pm 7.565
Post-Supplementation	41.75 \pm 8.892	48.88 \pm 8.501

Antioxidant function status was also examined on the same 16 subjects before and after two months of vitamin C or placebo supplementation using cultured lymphocytes and exposure to an oxidative stress (cumene hydroperoxide). No differences were found between treatment groups at baseline (t-test, $p = 0.37$) or at follow-up ($p = 0.57$). In addition, no differences were found between baseline and post-supplementation for either treatment group (paired t-test, $p = 0.97$) for the vitamin C group and $p = 0.08$ for the placebo group). Finally, a t-test showed no difference between the amount of change in the placebo versus the vitamin C group ($p = 0.12$). Thus, vitamin C supplementation had no apparent beneficial effect on antioxidant function.

Study Sub-Hypotheses

Sub-Hypothesis 1: High Stress Level is Predictive of URTI in Marathon Runners

Stress levels were determined using a three-part questionnaire (Cohen et al. 1991) at baseline ($n = 91$; one runner did not complete the baseline stress questionnaire) and at one week pre-marathon ($n = 68$). Stress levels were determined using values from Cohen et al. (1991), with level one as the lowest stress level and level four as the highest (see Table 13). Each of the three stress scales was considered individually and also as a composite stress index by summing the stress level scores. Baseline and follow-up scores were compared for differences using McNamara's Test for paired comparisons ($n = 67$). STAT/XACT was used since SAS cannot perform this test. The stress questionnaire contained three parts; no differences between baseline and follow-up were found for Mood ($p = 0.54$) or Perceived Stress ($p = 0.33$). However, a significant difference was found between baseline and follow-up for Life Events ($p = 0.002$). Life Events scores tended to result in lower stress level scores (less stress) at follow-up compared to baseline (54.6% of the subjects fell into the bottom two levels at follow-up, compared to 42.8% at baseline). Thus, only the baseline results were used for Mood and Perceived Stress, whereas baseline and follow-up were considered for Life Events. Similarly, no difference was found between baseline and follow-up for the composite stress index (McNamara's test, $p = 0.64$, $n = 67$).

Repeated measures analysis of variance was used to examine whether there were any treatment or group differences in stress levels during the study. Running group was significant for each of the three stress indicators except for Life Events at baseline ($p = 0.01$ for perceived stress at baseline, $p = 0.005$ for mood at baseline, $p = 0.22$ for life events at baseline, $p = 0.01$ for life events at follow-up), with more runners tending to fall into the lower stress levels than the sedentary subjects. (No treatment or treatment-by-running group differences were found for any of the stress indicators). However, no significant differences were found for the composite stress index (repeated measures ANOVA, running group $p = 0.29$, treatment $p = 0.16$, treatment-by-running group interaction $p = 0.24$).

As shown below in Table 13, subjects with higher stress scores were not at greater risk of cold symptoms. Stress levels were entered into the preliminary step-wise logistic regression models and since stress levels were not significant (p-values ranged from 0.36 to 0.75 for the individual stress scales and 0.10 to 0.57 for the composite stress index), they were not included in the logistic regression.

Sub-Hypothesis 2: High Training Mileage is Predictive of URTIs in Marathon Runners.

Multiple logistic regression analysis was used to determine whether training mileage was related to URTIs. The average running mileage was determined based on running logs for the entire 12 weeks of the study. Running mileage was not predictive of URTI when this variable was added to the regression model reported for marathon runners above (parameter estimate 0.0012, odds ratio 1.001, p = 0.96).

When this variable was entered alone, along with treatment and adjusted for month, average running mileage was again not predictive of URTIs (parameter estimate -0.00173, odds ratio 0.998, p = 0.92).

Upon closer examination of the running data, running mileage was noted to be on the low side for marathon runners (median 46.8 km or 29 miles per week, 75th percentile = 56.5 km or 35 miles per week). When the runners were divided according to running mileage, eleven cases of URTIs (35.5% of subjects) were reported among runners who ran under 56.5 km (35 miles) per week and 5 cases (38.5% of subjects) were reported among runners who ran over 56.5 km (35 miles) per week.

TABLE 13. Stress Levels (Life-Events, Perceived Stress and Mood per Cohen et al. 1991) and Incidence of Reported URTIs (number of subjects in each stress level, number of subjects with URTIs, and % of subjects with URTIs) for Marathon Runners (n = 43) and Sedentary Subjects (n = 48)*.

Stress Level [†]	Marathon Runners			Sedentary Subjects		
	n	# with URTIs	%	n	# with URTIs	%
----- Life-Events at Baseline -----						
1	5	14	35.7	7	12	58.3
2	5	16	31.3	5	16	31.3
3	5	9	55.6	3	10	30.0
4	1	4	25.0	3	10	30.0
----- Life-Events at Follow-Up -----						
1	4	15	26.7	2	8	25.0
2	6	14	42.9	5	16	31.3
3	0	3	0.0	0	4	0.0
4	0	1	0.0	4	7	57.1
----- Perceived Stress at Baseline -----						
1	5	15	33.3	4	13	30.8
2	6	15	40.0	7	13	53.8
3	2	5	40.0	3	10	30.0
4	3	8	37.5	4	12	33.3
----- Mood at Baseline -----						
1	3	14	21.4	4	6	66.7
2	5	8	62.5	6	13	46.2
3	2	8	25.0	6	15	40.0
4	6	13	46.2	2	14	14.3

[†] stress level of 1 represents the lowest stress level and 4 represents the highest stress level.

* n = 33 Runners and 35 Sedentary for Life-Events at Follow-Up.

TABLE 14. Composite Stress Index (Sum of Levels for Three-Part Stress Scale per Cohen et al. 1991) and Incidence of Reported URTIs (number of subjects in each stress level, number of subjects with URTIs, and % of subjects with URTIs) for Marathon Runners (n = 43 at baseline and 33 at follow-up) and Sedentary Subjects (n = 48 at baseline and 35 at follow-up).

Stress Level [†]	Marathon Runners			Sedentary Subjects		
	n	# with URTIs	%	n	# with URTIs	%
----- Composite Stress Index at Baseline -----						
1	4	15	26.7	6	10	60.0
2	5	11	45.5	6	18	33.3
3	6	13	46.2	1	5	20.0
4	1	4	25.0	5	15	33.3
----- Composite Stress Index at Follow-Up -----						
1	5	15	33.3	4	11	36.4
2	1	6	16.7	0	6	0.0
3	3	8	37.5	1	6	16.7
4	1	4	25.0	6	12	50.0

* stress level of 1 represents the lowest stress level and 4 represents the highest stress level.

Sub-Hypothesis 3: Training Intensity (Pace) is Predictive of URTIs in Marathon Runners.

Average running pace throughout the study was determined based on the running logs for the 12 weeks of the study. The median running pace was 11.1 km per hr (6.86 mph), with 50% of the subjects running between 10.2 and 11.9 km per hr (6.3 mph and 7.4 mph). When the subjects were divided into two groups based on median running pace, there were 9 cases of URTIs (36.0% of subjects) among runners who ran under 11.3 km per hr (7 mph), and 7 cases (36.8% of subjects) among runners who ran above 11.3 km per hr (7 mph). None-the-less, logistic regression revealed a significant relationship between pace and URTIs ($p = 0.04$) (see logistic regression above).

Dietary Analysis

Estimated usual dietary intake over the past year was determined at baseline (immediately prior to intervention) on 135 subjects using the Health Habits and History Questionnaire, a semi-quantitative food frequency questionnaire. Six subjects were excluded from the dietary analysis due to questionable accuracy of their responses (see Chapter 3 for detailed methodology).

ANOVA was performed using the log of actual nutrient intakes due to unequal variances in the untransformed values. In order to avoid performing logs on zeros, the log ($x + 1$) was used for supplemental vitamin C and the log ($x + .1$) was used for alcohol. Untransformed dietary data is shown in the tables below for ease of interpretation.

ANOVA was run for dietary, supplemental, and total intake of vitamin C to determine whether there were any differences due to running group, treatment, or gender (see Table 15). Supplemental vitamin C refers to self-selected vitamin C supplementation prior to study intervention. For the most part, only the significant findings are mentioned below. Although no significant difference was found for treatment group (vitamin C versus placebo), a treatment-by-gender interaction was found for dietary vitamin C ($p = 0.03$) (mean \pm SEM = 153 ± 13.0 mg and 176 ± 32.5 mg for placebo-treated males and females respectively, and 229 ± 24.4 mg and 149 ± 24.6 mg for vitamin C-treated males and females, respectively). Thus, the placebo-treated females had a higher intake of vitamin C than the placebo-treated males, whereas the vitamin-C treated males had a higher intake of vitamin C than the vitamin C-treated females. ANOVA of total vitamin C intake (supplemental plus dietary intake) revealed a significant difference between the marathoners and the sedentary subjects for total vitamin C intake ($p = 0.02$). As shown on Table 15, the marathon

TABLE 15. Mean Estimated Usual Dietary Intake of Vitamin C, Supplemental Intake of Vitamin C, and Total Intake of Vitamin C (mg/d) for the Year Prior to the Study for Vitamin C and Placebo-treated Runners and Sedentary Subjects⁺ (Mean \pm SEM).

Vitamin C Intake	Runners		Sedentary	
	Vitamin C n = 41	Placebo n = 30	Vitamin C n = 29	Placebo n = 29
Dietary [*]	207 \pm 20.5	169 \pm 20.5	210 \pm 37.7	149 \pm 14.9
Males	219 \pm 23.8	155 \pm 19.1	248 \pm 53.1	151 \pm 17.2
Females	160 \pm 34.9	238 \pm 75.8	139 \pm 35.7	145 \pm 29.5
Supplemental	234 \pm 66.1	209 \pm 94.5	102 \pm 54.4	78 \pm 39.4
Males	251 \pm 78.6	223 \pm 112.3	139 \pm 82.3	88 \pm 58.5
Females	168 \pm 103.7	138 \pm 93.6	31 \pm 9.7	58 \pm 30.2
Total ^{**}	442 \pm 71.4	378 \pm 94.5	312 \pm 66.8	227 \pm 41.9
Males	470 \pm 83.2	379 \pm 112.5	387 \pm 96.3	239 \pm 57.5
Females	328 \pm 128.1	377 \pm 93.5	171 \pm 39.7	203 \pm 56.1

⁺ n = 33 vitamin-C treated male runners, 25 placebo-treated male runners, 19 male vitamin C-treated sedentary males, 19 placebo-treated sedentary males, 8 vitamin-C treated female runners, 5 placebo-treated female runners, 10 female vitamin C-treated sedentary females, 10 placebo-treated sedentary females.

^{*} Analysis of variance revealed a significant treatment-by-gender interaction for dietary vitamin C intake, p = 0.03.

^{**} Analysis of variance revealed a significant difference between marathon runners and sedentary subjects for total intake of vitamin C, p = 0.02.

TABLE 16. Mean Estimated Energy (kcalories) and Macronutrient Intake for the Year Prior to the Study in Male and Female Marathon Runners and Sedentary Subjects (Mean \pm SEM).

	Runners Males n = 58	Females n = 13	Sedentary Males n = 38	Females n = 20
Energy (kcalories/d) [#]	2179 \pm 115	1776 \pm 220	2520 \pm 195	1634 \pm 204
Carbohydrate (gm/d)	252 \pm 12.9	230 \pm 29.8	263 \pm 19.2	188 \pm 22.2
Protein (gm/d)	88 \pm 4.5	69 \pm 9.4	102 \pm 7.8	67 \pm 7.4
Fat (gm/d)	87 \pm 6.0	64 \pm 10.2	113 \pm 10.1	66 \pm 11.1
Carbohydrate (%) ^{* +}	47.1 \pm 1.1	51.2 \pm 2.7	42.6 \pm 0.9	46.7 \pm 1.6
Protein (%) ⁺⁺	16.5 \pm 0.3	15.6 \pm 0.8	16.3 \pm 0.3	17.1 \pm 0.9
Fat (%) ^{**}	34.9 \pm 1.0	31.9 \pm 2.4	39.1 \pm 1.0	34.9 \pm 1.8
Alcohol (%)	3.5 \pm 0.6	2.5 \pm 0.8	3.3 \pm 0.7	2.2 \pm 1.2

* Runners versus sedentary (ANOVA) p = 0.01.

** Runners versus sedentary (ANOVA) p = 0.01.

Gender difference (ANOVA) p = 0.003).

+ Gender difference (ANOVA) p = 0.02.

++ Gender difference (ANOVA) p = 0.001.

runners had a higher total intake of vitamin C compared to sedentary subjects.

Energy and macronutrient intake are shown in Table 16 (untransformed data is shown for ease in interpretation). The analysis of variance (ANOVA) revealed that marathoners consumed a higher percentage of energy as carbohydrates ($p = 0.01$) and a lower percentage of energy as fat ($p = 0.01$) compared to the sedentary group. However, energy intake was similar between the marathoners and the sedentary subjects ($p = 0.95$). No differences were found in energy or macronutrient intake between vitamin C and placebo-treated subjects.

Gender differences were found for the percentage of carbohydrate intake ($p = 0.02$) and for kcalorie intake ($p = 0.003$) (see Table 16), with males having higher intakes than females. No gender differences were found for percentage of protein intake ($p = 0.51$), while a gender difference was found for protein intake ($p = 0.001$). Again, males had a greater intake than females.

Chapter Five
Discussion and Conclusions

Main Study Hypothesis

Hypothesis 1: Vitamin C Supplementation Decreases the Risk of Upper Respiratory Tract Infections (URTIs) among Marathon Runners

The major finding of this study was that vitamin C supplementation did not reduce the incidence of colds in marathon runners compared to placebo-treated runners during the two months prior to and one month following a marathon. In fact, the runners who received vitamin C supplements tended to have a slightly greater duration and severity of colds compared to placebo-treated runners, although this finding was not statistically significant.

Similarly, vitamin C supplementation did not reduce the incidence of colds among the sedentary subjects. However, the sedentary subjects who received vitamin C showed reduced severity ($p = 0.04$) and also showed a trend toward lowered duration (not significant) of colds.

Our finding that vitamin C supplementation had no effect on the incidence of colds is in agreement with the vast majority of the literature (Anderson 1972, Chalmers 1975, Thomas and Holt 1978, Pitt and Costrini 1979, Hemila 1992, Hemila 1994). In general, vitamin C appears to play little role in reducing the incidence of colds, with the possible exception in cases of severe stress (Hemila 1992, Peters et al. 1993, Bendich and Langseth 1995), whereas it does seem to modify cold symptoms and severity (Anderson 1972, Hemila 1994, Hemila and Herman 1995).

Possibly, the runners in the present study were not under sufficient physiological stress in order to show any benefit of vitamin C supplementation. If this is the case, however, then anyone who is less stressed or less active than the average marathon runner is also unlikely to reduce risk of colds with vitamin C supplementation.

Generally, the literature has shown a benefit in reduced symptoms and severity with vitamin C supplementation (Anderson 1972, Hemila 1994, Hemila and Herman 1995), but this benefit is rather moderate and may not have been large enough to have shown up in the present study. In fact, some authors have described this benefit of vitamin C supplementation as marginal at best and not worth supplementing for (Chalmers 1975, Pitt and Costrini 1979). Others, however state that the risks of vitamin C supplementation are negligible and the benefits, including chronic disease prevention as well as possible effects against the common cold, may warrant supplementation (Gershoff 1993, Enstrom 1993, Hemila 1994b). None-the-less, no recommendation in favor of vitamin C supplementation can be made based on the present study for marathon runners or for sedentary subjects in terms of preventing the common cold.

Our findings are in contrast with those of Peters et al. (1993) who found that vitamin C supplements reduced the incidence of URTIs by 50% in ultramarathoners. However, the ultramarathoners had a much greater incidence of cold symptoms (33% in the vitamin C group and 68% in the placebo group during the two weeks following an ultramarathon) and a larger sample size. Peter et al. found no differences in the duration of symptoms between the vitamin C-treated runners and the placebo-treated runners, which is contrary to most of the literature on

vitamin C. In addition, the incidence of colds among non-runners was extraordinarily high. In fact, there were more colds among vitamin C-supplemented non-runners (53% of subjects) than vitamin C-supplemented runners (33% of subjects). However, the duration of cold symptoms was shorter in the vitamin-C supplemented non-runners compared to placebo-treated non-runners (4.2 ± 2.1 versus 5.6 ± 3.2 days)(mean \pm SD).

Still the findings of Peters et al. are surprising since they provided only 600 mg of vitamin C, did not control additional vitamin C supplementation, and relied on subject recall of cold symptoms in a telephone interview two weeks following the ultramarathon to determine incidence of URTIs. On the other hand, the ultramarathoners ran greater distances than the marathoners in the present study, and participated in a winter race, which may have made them more prone to URTIs. In addition, the higher stress of training among the ultramarathoners may have increased the likelihood of benefiting from supplemental vitamin C.

Interestingly, in the present study seven runners (15.9) and four sedentary controls (8.3%) reported URTI symptoms during the two weeks following the marathon. While not significantly different, this finding suggests the possibility of increased cold symptoms following the marathon, which is in agreement with other studies of marathon and ultramarathon runners (Nieman et al. 1990, Peters et al. 1983, Peters 1990). For example, Nieman et al. (1990) found that 12.9% of marathoners had cold symptoms during the week following the Los Angeles Marathon, while 9.1% of the runners in the present study reported cold symptoms during the week following the marathon. Nieman et al. (1990) reported that 43.2% of the marathoners had URTIs during the two months before the marathon, which is similar to our findings (33.3% of the vitamin C-treated runners and 42.9% of the placebo-treated runners had cold symptoms during the three month study). The finding that the runners in the present study were not overtraining may also have reduced the likelihood of finding any benefits with vitamin C supplementation.

Logistic regression revealed an increased risk of colds with increasing age. The average age of the study subjects was 43 years. Logistic regression also revealed a reduced risk of colds with increased alcohol intake. However, the majority of the subjects (82.6%) reported drinking less than one drink per day, and only 4.4% reported drinking more than 2 drinks per day. Therefore, this finding does not imply that drinking large amounts of alcohol is of any benefit.

Hypothesis 2: Vitamin C Supplementation Increases Plasma Vitamin C Levels

Marathoners had higher plasma vitamin C levels than sedentary controls throughout the study ($p = 0.0001$). In addition, a treatment-by-running group effect was found for plasma vitamin C ($p = 0.03$), whereas treatment alone was not significant ($p = 0.10$). Thus, a treatment effect was found, but must be considered in light of an interaction with the running group. This is due to the fact that the

runners had higher plasma vitamin C levels compared to the sedentary subjects at baseline (prior to intervention), and that vitamin C levels tended to increase in the vitamin C-treated runners and decreased in the placebo-treated runners whereas vitamin C levels tended to increase in the vitamin C-treated sedentary subjects and remained the same in the placebo-treated sedentary subjects. None-the-less, the increase in plasma vitamin C levels with supplementation were somewhat less than expected, which may be due to the following reasons: 1) baseline levels may have been inflated due to prior vitamin C supplementation since subjects were restricted to 200 mg per day upon entry into the study but supplementation prior to baseline was not controlled, 2) running is known to temporarily reduce plasma vitamin C levels (Garry and Appenzeller 1983, Boddy et al. 1974, Gleeson et al. 1987) and at least some runners had their one-week pre-marathon blood sample drawn immediately following their morning runs, which may have lowered the values for this time point, 3) again, running is known to reduce plasma vitamin C levels for at least two days (Gleeson et al. 1987) and the post-marathon levels, which were drawn two days following the race, may have remained lower than normal.

Looking at Figure 1, it is clear that vitamin C supplements did raise vitamin C levels in both runners and sedentary controls, whereas those subjects receiving placebo tablets had either no change (sedentary controls) or lower plasma vitamin C levels towards the end of the study (runners). Again, the higher baseline value in the runners probably reflects their use of supplements prior to the study.

Hypothesis 3: Vitamin C Supplementation has no Effect on Lymphocyte Proliferation in Marathon Runners

Vitamin C supplementation had no apparent effect on lymphocyte proliferation or on antioxidant function. This result was somewhat surprising as some studies have shown that vitamin C enhances lymphocyte proliferation (Fraser et al. 1978, Anderson et al. 1980, Kennes et al. 1983). However, other studies have found no effect (Jacob et al. 1991, Kay et al. 1982, Delafuente et al. 1986). Thus, vitamin C supplementation appears to augment lymphocyte proliferation only in certain cases, perhaps due to differences in mitogens used, cell populations studied, differing amounts of vitamin C used, or differences in populations studied (Delafuente et al. 1986, Shive 1988). For example, phytohemagglutinin (PHA) and Concanavalin-A (Con-A) tend to activate T-lymphocytes, while lipopolysaccharides tend to activate B-lymphocytes and pokeweed tends to stimulate both T- and B-lymphocytes (Shive 1988).

In the present study, Spectra Cell Laboratories used PHA for determination of lymphocyte proliferation. Another important difference between studies is that some researchers added supplemental vitamin C in vitro, whereas in the present study the vitamin C was given orally to the subjects. Still, Anderson et al. (1980) reported stimulation of lymphocytes with PHA and Con-A with daily ingestion of 1, 2, and 3 gm of vitamin C in five healthy adults. However, Anderson

et al. (1980) used much higher doses of mitogens than Spectra Cell Laboratories (25 and 50 ug/ml compared to 2 mg/L or 2 ug/ml).

Study Sub-Hypotheses

Sub-Hypothesis 1: High Stress Level is Predictive of URTIs in Marathon Runners

We did not find any effect of stress level on incidence of URTI symptoms. This was somewhat unexpected given our use of a questionnaire which has been previously been used with success to determine risk of acquiring URTIs when subjects were inoculated with a cold virus (Cohen et al. 1991). However, Cohen et al. (1991) used a large sample size to find this effect (n = 394). In addition, overall incidence of URTIs in the present study was somewhat low, as over 60% of the subjects remained healthy throughout the study, which may have reduced the likelihood of finding any effects of stress on cold symptoms.

Our finding of no relationship between stress level and cold symptoms is in contrast with Nieman et al. (1990) who reported a significantly higher incidence of cold symptoms among runners in the high stress group (45.2% of the subjects in the high stress group reported cold symptoms compared to 36.3% of subjects in the low stress group). However, the implication of this finding is unclear since they reported that more of the higher distance runners were also in the higher stress group.

Sub-Hypothesis 2: High Training Mileage is Predictive of URTIs in Marathon Runners

No relationship was found between training mileage and risk of cold symptoms, which is in contrast with other studies (Heath et al. 1991, Nieman et al. 1990). For example, Nieman et al. found a two-fold increase in risk of URTIs in runners that ran more than 97 km (60 miles) per week compared to runners who ran under 32 km (20 miles) per week. Our sample size may have been too small to find such an effect (there were 2,016 runners in the study by Nieman et al.). In addition, running mileage was noted to be rather low for marathon runners in the present study (median 47 km or 29 miles, 75th percentile = 56 km or 35 miles). Nieman et al. (1989a) proposed a U-shaped curve for the risk of acquiring colds versus exercise level, where both very low and very high levels of exercise compromise immune function while moderate exercise enhances immune function. Thus, rather than overtraining and compromising their immune status, the amount that the subjects in the present study were running may have been enough to improve their immune function.

Sub-Hypothesis 3: Training Intensity (Pace) is Predictive of URTIs in

Marathon Runners

Logistic regression showed that a faster average training pace during the 12 weeks of the study, as determined by daily running logs, was significantly related to risk of cold symptoms ($p = 0.04$). As noted above with running mileage, the marathon runners did not appear to be training excessively hard. The median running pace was 11.1 km per hr (6.86 mph), with 50% of the subjects running between 10.2 and 11.9 km per hr (6.3 mph and 7.4 mph).

Additional factors among the marathon runners which were related to risk of cold symptoms included the number of marathons previously run, marathon personal best time, and the distance of the longest run of the week. Specifically, the more marathons previously run, the longer the marathon personal best, and the shorter the distance of the longest run of the week were related to increased risk of URTIs.

In contrast, Nieman et al. (1990) reported that runners who had fewer years of running experience were at greater risk of colds ($p < 0.05$). However, Nieman et al. (1990) also found that runners who ran fewer miles per week (N.S.) and (as in the present study) ran a shorter distance during the average longest weekly run had a greater risk of URTIs ($p < 0.05$).

Limitations of the Study

Some of the major limitations of the study included the relatively small sample size and also the loss of many randomized subjects before the onset of the study (a total of 92 subjects completed the study; 44 marathon runners and 48 sedentary controls) compared to larger studies by Nieman et al. (1990) with 2,016 marathon finishers, Heath et al. (1991) with 530 runners, and Peters et al. (1993) with 92 ultramarathon runners. In addition, the present study included a relatively small number of female subjects (25% of runners were female). However, the number of female subjects reflected the overall percentage of women who ran the marathon, with 21.2% of the finishers being female.

Another important limitation to this study pertains to the amount of training by the runners. As revealed by the running logs and the running history questionnaires, the marathon runners were clearly not overtraining. They averaged 53 km (33 miles) per week during the year prior to the study, had a marathon personal best of 3 hours and 23 minutes, and had previously run an average of 17 marathons. Thus the benefits of studying runners in a major marathon as opposed to a local race should be considered in future research efforts.

By comparison, the marathon runners in the study by Nieman et al. (1990) ran greater distances per week (61 km or 38 miles). In addition, with the substantially larger sample size, Nieman et al. were in a better position to determine the influence of running mileage on risk of URTIs. Since part of the theory behind this study was that overtraining and running a marathon would make the runners more susceptible to URTIs, the finding that the marathon runners were not training excessively may explain the lack of beneficial effects of vitamin C on preventing URTIs. In fact, the only cases in the literature where vitamin C has been demonstrated to reduce

the incidence of colds are when the subjects were under severe stress, as with Canadian military troops during Arctic exercises (Hemila 1994), ski school students in the Swiss Alps (Hemila 1994), and ultramarathon runners (Peters et al. 1993).

Another limitation of the study was that a considerable number of the subjects (68% of the placebo-treated controls, 57% of the vitamin C-treated controls, 57% of the placebo-treated runners, and 67% of the vitamin C-treated runners) never had any cold symptoms during the study period. This relatively low incidence rate was due to studying the subjects from mid-July through mid-October and not during the peak cold and flu season. Further, as with most of the studies to date on exercise and URTIs, the presence of URTIs was determined by self-report of cold symptoms. We attempted to include a clinical diagnosis by a nurse practitioner on a voluntary basis, but found that the subjects were unwilling to participate in this part of the study. Future studies should probably include this component, although a financial or other inducement may be necessary in order to gain adequate participation.

Conclusions

Findings

In summary, we found no apparent benefit of vitamin C supplementation on the incidence of URTIs, despite excellent compliance of the subjects with taking their supplements during the three month study. Vitamin C supplementation did not significantly affect the incidence of colds among marathon runners or sedentary subjects. In addition, vitamin C supplementation did not decrease the duration or severity of cold symptoms in the marathon runners, although it did reduce the severity of colds in the sedentary subjects. However, only about 40% of the study subjects succumbed to colds during the study and the marathon runners were noted not to be training excessively, which may have enhanced rather than impaired immune function.

Vitamin C supplements did raise plasma vitamin C levels, as expected, but not to the extent expected. This was thought to be due to a number of the runners taking vitamin C supplements prior to the onset of the study which would have inflated baseline levels. Vitamin C supplementation was restricted to 200 mg during the study, and a number of the marathon runners agreed to stop taking vitamin C supplements upon recruitment into the study. In addition, a temporary reduction of plasma vitamin C levels due to running may have occurred on the morning of the second blood drawing as a number of the runners were known to have run that morning prior to having blood drawn, and possibly the two day post-marathon levels were still somewhat below normal as vitamin C levels are known to remain low for as long as three days after running (Gleeson et al. 1987).

Vitamin C did not have any beneficial effect on lymphocyte proliferation or antioxidant function with the techniques used in this

study. The literature on the effects of vitamin C on lymphocyte proliferation is quite mixed and may be due to the use of various different mitogens, differing vitamin C doses, or differences between the populations studied.

Stress level as determined by the questionnaire of Cohen et al. (1991) did not appear to predict risk of URTIs. This finding was surprising as the study by Cohen et al. (1991) found a dose-response relationship between stress levels and cold incidence when subjects were inoculated with cold viruses. However, the detection of the relationship between stress and clinical illness required a large sample size (394 subjects, 27 to 47% of the subjects displayed clinical cold symptoms). So while stress level may be a factor, risk of URTIs in the present study was more strongly related to other factors, such as gender, number of marathons previously run, longest run per week, and average running pace.

In contrast with other studies, running mileage was not related to the risk of URTIs. Again, the moderate training of the subjects and the small sample size may have prevented finding an effect. However, a harder training intensity (running pace) was related to an increased risk of cold symptoms. In addition, the more marathons previously run, longer marathon personal records, and shorter longest runs of the week were predictive of URTIs. Female runners also had a greater risk of URTIs than male runners, which agrees with findings by Heath et al. (1991) who reported that male runners had fewer colds than female runners (odds ratio = 0.14, 95% confidence interval = 0.03 to 0.68) in a year long study, but contrasts with Neiman et al. (1990) who found no gender difference in risk of URTIs in marathon runners.

Among the sedentary controls, younger subjects had a lower risk of URTIs. In addition, sedentary subjects who drank alcohol had a lower risk of URTIs. However, alcohol intake among all of the subjects was quite low (83% of the subjects drank less than one drink per day), so this finding does not imply that large amounts of alcohol are beneficial.

In summary, we did not find any benefit of vitamin C supplementation on risk of colds in marathon runners. If vitamin C has any effect on incidence of colds, it is unlikely to be a large effect on a healthy and relatively active population. This finding is in agreement with the vast majority of literature on vitamin C which has found that while vitamin C can reduce the symptoms and severity of a cold somewhat, supplementation has no effect on incidence in the general population.

Further Research

Based on the present study, vitamin C appears to offer no benefit against the common cold in marathon runners. However, the current study was based on a group of New Mexican runners who were not over-training based on an average weekly mileage of about 33 miles per week in the year prior to the study. Thus these results should not be applied to people who are exposed to extraordinary amounts of physical

or psychological stress, such as elite athletes or ultramarathoners. Thus, future research on the effect of vitamin C on colds should focus on more highly stressed subjects, and ideally should include a larger sample size and fewer dropouts. In addition, the inclusion of a higher proportion of female subjects is desirable.

Other populations that warrant further study to determine whether vitamin C might improve resistance against the common cold or improve immunity include the elderly and the immune-compromised. For example, recent studies have shown improved resistance against colds in the elderly with a multivitamin/mineral supplement, but have not determined which nutrients might be responsible (Chavance et al. 1993, Chandra 1992, Meydani 1990, Penn et al. 1991, Meydani 1992). In addition, vitamin C supplementation might play a role in other respiratory conditions besides the common cold, such as in the treatment of asthma or bronchitis (Johnston et al. 1992, Schwartz and Weiss 1990, Bucca et al. 1989).

In conclusion, the vast majority of studies, including the present study, have shown that vitamin C does not prevent the common cold. We found no benefit of vitamin C supplementation (1000 mg per day) on incidence of URTIs in marathon runners.