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NAVAL SUBMARINE MEDICAL RESEARCH LABORATORY

SUBMARINE BASE, GROTON, CONN.



REPORT NUMBER 933
VITAMIN C STATUS OF SUBMARINERS
by
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Naval Medical Research and Development Command
Research Work Unit ZF51.524.004-9023

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ABSTRACT

Plasma vitamin C levels were determined for 28 submariners prior to, during, and after a 68-day patrol. A significant reduction occurred in plasma vitamin C levels during the patrol compared to levels obtained before and after the patrol. These reductions were unrelated to health and performance effects as determined by total dispensary visits, frequency of upper respiratory infections, and training accomplishments. Smokers had significantly lower levels of plasma vitamin C than non-smokers, as did those who had made several previous patrols. Vitamin supplements did not appear to ameliorate the vitamin C deficiency found among smokers, but did enhance the plasma levels of vitamin C among non-smokers. These findings indicate that dietary or supplemental intake of vitamin C among submariners should be increased, especially among those who smoke or who have made several patrols.

Although vitamin C was not identified biochemically until this century, the importance of fresh fruits and vegetables containing high levels of vitamin C in the maintenance of health and performance has been recognized for over 400 years. While exploring the New World during the 15th and 16th centuries, entire crews were ravaged by scurvy following prolonged periods at sea without fresh food (1). In 1753, a Scottish physician named James Lind published data showing that consumption of oranges and lemons could cure scurvy among sailors (2). In 1804, the Royal Navy required that sailors consume a daily ration of lemons or limes (hence, the name "limey"). Research over the last 50 years has isolated vitamin C biochemically, and demonstrated that humans are totally dependent on diet as the sole source of vitamin C because of genetic deficiency in the enzyme that catalyzes the oxidation of L-gluconic acid to L-ascorbic acid (3, 4).

Today, fresh fruits and vegetables are available routinely onboard most Navy ships. Some ships, however, do not have regular access to fresh food stores because the mission of these ships requires that they remain undetectable, thereby preventing resupply at sea. These ships are nuclear submarines on deterrent ballistic missile patrols. Despite the historical involvement that navies have had in recognizing the importance of vitamin C in health, little has been done to determine if prolonged submarine patrols results in significant vitamin C depletions. During these patrols, that last from 60 to 75 days, fresh fruits and vegetables are consumed during the first week or two, and are not replenished until the patrol is completed.

Vitamin C mediates the health effects described above through regulation of a number of biochemical processes. Through regulation of these processes, vitamin C has been implicated in the frequency and severity of respiratory

infections, notably the common cold (5, 6). Collagen synthesis is another important process found to depend heavily on the availability of vitamin C (7-11). Deficiencies of vitamin C result in problems in wound-healing (i.e., failure of wounds to heal or deterioration of previously healed wounds) that have been attributed directly to impaired collagen formation (7, 10, 11). Submariners may therefore be a population at risk for wound-healing problems because, as mentioned above, access to dietary vitamin C is diminished substantially during extended submarine patrols.

Research has also shown that smokers have low levels of vitamin C (12, 13). A possible explanation for this effect is that smokers absorb less vitamin C from the gut than non-smokers. These findings indicate that smokers may require twice as much vitamin C intake as non-smokers to maintain comparable blood level of L-ascorbic acid. These data have not been replicated under field conditions, however, nor has the relationship of this condition to health and performance been determined.

The following research will document the plasma vitamin C levels of a typical submarine crew, compare these levels between smokers and non-smokers, and determine whether or not these levels are related to respiratory infections, wound-healing, overall health and training accomplishments.

METHOD

Twenty-eight men from a ballistic missile nuclear submarine crew of 155 volunteered to participate. The average age of the men was 25.2 years, with a range of 19 to 36 years. The group consisted of two officers, four midshipmen (officer candidates) and 22 enlisted men. The median number of patrols made by this group was three (ranging from one to seventeen), including the present patrol. The volunteers were in excellent health and did not have a history of major disease. Blood samples were collected two days before

submerging (pre-patrol sample), on day 63 of the 68-day patrol (late-patrol sample), and 38 days after returning from patrol (post-patrol sample).

Pre-patrol and late-patrol plasma samples were deprotenized and oxidized on the submarine prior to shipment on dry ice for vitamin C determinations. Post-patrol samples were collected and prepared for analysis at the laboratory about five weeks after the submariners had completed the patrol. Plasma specimens were assayed for vitamin C within one week of collection using a modification of the 2,4-dinitrophenylhydrazine method of Roe and Kuether (14).

Before each sampling period, the volunteers were asked to complete a questionnaire that asked for information about diet, use of vitamin supplements, cigarette consumption per week, and whether or not they had problems with wounds healing more slowly than normal. Sick call records were maintained during the entire patrol by the hospital corpsman assigned to the ship. The group made an average of 1.42 sick call visits during the patrol (ranging from none to ten visits). These records were analyzed further for sick call visits involving symptoms of respiratory infection (congestion, coughing, sore throat, earache, and so forth).

RESULTS

Table 1 shows the means and standard errors for plasma vitamin C levels of the twenty-eight submariners two days before the patrol (pre-patrol) on Day 63 of the 68-day patrol (late patrol), and 38 days post-patrol (post-patrol). A one-way analysis of variance for repeated measures showed that significant differences in plasma vitamin C levels existed across the three test conditions ($F = 4.29$, $df = 2, 81$; $p < .05$). Subsequent t -tests demonstrated that the late patrol vitamin C levels differed significantly from both pre-patrol and post-patrol levels (pre-patrol $\times$ late patrol: $t = 2.24$; $p < .05$; post-patrol $\times$ late patrol: $t = 3.35$; $p < .01$). A significant difference was not found between

pre-patrol and post-patrol vitamin C levels ($t = 1.57$; $p > .05$). These results indicate that during the late-patrol period, plasma vitamin C levels were significantly lower than during the control (pre- and post-patrol) periods.

From the above data, the 28 submariners were divided into two equal groups--the 14 who had the highest levels and the 14 who had the lowest levels of plasma vitamin C during the late patrol period. The range for the high group was 0.87 to 2.32 mg/dl ($\bar{M} = 1.46$ mg/dl), while the range for the low group was 0.14 to 0.72 mg/dl ($\bar{M} = 0.43$ mg/dl). With only one exception, the levels for the low group were below the minimum normal level of 0.60 mg/dl defined by Nino and Shaw (4). Comparisons were then made between these two groups for wound-healing problems, sick call visits for respiratory infections, overall sick call visits and training performance. From research cited above, a priori predications were made that the low group would have more wound-healing problems and sick call visits, as well as fewer accomplishments, than the high group. Comparisons were made using t-tests for independent samples or z-tests for proportions.

For wound-healing, adjustments were made in group totals because three members of the low group and one member of the high group did not incur wounds (cuts or scratches) during the patrol. With these adjustments, seven members of the high group (53.8%) and two members of the low group (18.2%) reported that wounds took longer than normal to heal. This difference, which was in the unexpected direction, was significant ($z = 1.798$; $p < .05$).

Overall sick call visits were not found to differ significantly between the two groups. Nine members of the low group (64.3%) and seven members of the high group (50%) made at least one visit to the dispensary for some type of illness or injury during the patrol ($z = 0.748$; $p = \text{NS}$). If only those dispensary visits involving symptoms of respiratory infection were analyzed, six members of the low group and three members of the high group were found to make at least one visit to the dispensary for this purpose. Again, the

difference was not significant ($z = 1.193$; $p = \text{NS}$).

Training accomplishments were rated according to difficulty. Completing minor training programs such as correspondence courses, an onboard college course (in business math) or watch section qualifications were rated as "1", while completion of major training programs such as qualification for the submarine insignia, chief of the watch or diving officer of the watch were scored as "2". Minor training could be completed normally in less than a single patrol, while major training normally required two or three patrols to complete. The findings showed that the low group completed an average of 1.96 units of training while the average for the high group was 3.32 units. The difference was statistically significant ($t = 1.878$; $p < .05$). While this difference may appear to be a crucial finding, subsequent data analysis indicated that factors other than vitamin C may be involved in the effect. A comparison of the ages of the two groups showed that the low group had an average age of 26.0 years and the high group had a mean age of 24.36 years. The difference of over one and one-half years, while not significant, indicated that the low group may have had more years of submarine experience than the high group. A comparison of mean years of submarine experience between the groups showed that the low group had been assigned to submarines an average of 3.61 years, while the high group had 1.68 years of experience. This difference was significant ($t = 2.413$; $p < .01$). Inasmuch as more experienced crew members are more likely to have completed major training during the first two years of submarine duty, the training differences between the two groups noted above may indicate that members of the low group did not complete as many training units as the high group because they had already completed the major training programs and many of the minor programs prior to this patrol. The results do, nonetheless, imply that late patrol vitamin C levels deteriorate with submarine experience. Inasmuch as other results presented above show that overall plasma vitamin levels for both groups combined well within the normal range prior to

the patrol, these latter findings indicate that more experienced submariners may be restricting dietary intakes of vitamin C during patrol.

In keeping with previous research cited above, smoking was found to be related to lower plasma vitamin C levels. Of the 28 crew members in both groups, eight smoked 10 or more cigarettes a day, and 20 were non-smokers (or smoked only a couple of cigarettes a week). The average late patrol plasma vitamin C level for the smokers was 0.525 mg/dl, while the mean level for the non-smokers was 1.115 mg/dl. This difference was highly significant ($t = 2.511$; $p < .01$). In addition, six of the smokers were in the low group and two in the high group. This difference was nearly significant ($z = 1.673$; $p < .10 > .05$).

Many of the crew members were found to take a vitamin supplement regularly during the patrol. This supplement, which contains 75 mg of ascorbic acid, is manufactured under a federal stock number and was issued ad libitum by the hospital corpsman onboard the submarine. Inasmuch as the above findings demonstrate that smoking may impair absorption of ascorbic acid, comparisons were made separately for smokers and non-smokers. Among non-smokers, those who took at least one vitamin supplement a day ($N = 6$) had a mean plasma vitamin C level during the late patrol period of 1.522 mg/dl, while those who did not take vitamin supplements ($N = 13$) had an average level of 0.968 mg/dl. This difference was significant ($t = 1.972$; $p < .05$). Of the eight crew members who smoked at least 10 cigarettes a day, only two were found who took vitamin supplements. While a statistical comparison involving a group this small would be unreliable, a review of the data showed that both of these crew members had a plasma vitamin C level of only 0.290 mg/dl. The mean vitamin C level of the six crew members who smoked and did not take vitamin supplements was 0.603 mg/dl. These findings indicate that while vitamin supplements of this type appear to enhance plasma level of vitamin C, such an effect may be substantially reduced among those who smoke 10 or more cigarettes a day.

DISCUSSION

The findings presented above show that modern submariners on extended patrols experience significant reductions in plasma vitamin C levels. In many cases, these reductions are below the minimum normal level. The lowest levels were found among smokers and those who had made the largest number of previous patrols. Reductions in plasma vitamin C, however, were unrelated to overall illness rates, including symptoms associated with upper respiratory infections. In addition, these reductions did not appear to be associated with performance effectiveness as documented by training accomplishments. Unexpected results were found for wound-healing--those who had the highest levels of plasma vitamin C reported having more trouble with wounds healing properly than those who had the lowest plasma levels of vitamin C.

The findings of wound-healing appear to contradict previous evidence showing that vitamin C is necessary in the formation of collagen, a key substance in the wound-healing process (7-11). A possible explanation of this unexpected finding is that the self-reported data were biased and therefore invalid. The high plasma vitamin C group may have been more conscious of health problems in general (perhaps even mildly hypochondriacal) than the low plasma vitamin C group, and perceived the healing process as taking longer. That health behavior (and perhaps perceptions) differed between the two groups is shown by the low frequency of smoking among members of the high group, as well as the routine use of vitamin supplements among this group. The ambiguity surrounding these results can be resolved only by analyzing in more detail the formation and sloughing of scabs during the patrol and baseline periods.

The data on health performance may also have been too gross to detect impairments, if needed such impairments existed. The results for upper respiratory infections were in the expected direction, however, with twice as many of the low group showing symptoms of the common cold than the high group. The similarities in overall illness and injury rates between the two groups

may simply represent the common risks assumed by the members of two groups in a crowded and self-contained environment. The performance results also show a need for better controlled and refined measures. The possibility that the low group may not have accomplished as many training units as the high group because they may have done so during earlier patrols makes the differences obtained between these two groups equivocal. The use of personnel who have never made a previous patrol would have provided better control over the training criterion. In addition, perhaps more discrete measures of performance involving manual, cognitive and vigilance skills would be appropriate.

The finding that those who made more patrols had lower levels of plasma vitamin C than those with less submarine experience may indicate that at least part of the reduction is related to dietary behavior. Perhaps those who had made several patrols reduced consumption of canned fruits and vegetables because they found these foods to be unappetizing, thereby lowering plasma vitamin C substantially. This effect, however, is complicated by the number of moderate to heavy smokers found in the low group. The data showing that smokers had lower levels of plasma vitamin C than non-smokers replicates previous findings (12, 13). In addition, the present findings indicate that vitamin supplementation involving 75 mg of vitamin C a day does not ameliorate this condition. This latter finding is consistent with previous data showing that smokers require twice as much vitamin C intake as non-smokers to maintain comparable levels of plasma vitamin C (12, 13).

The results described above demonstrate that submariners show substantial reductions in plasma vitamin C, and that at least part of this reduction can be attributed to smoking. Dietary restrictions of vitamin C may be involved as well. Inasmuch as previous research has shown that vitamin C mediates wound-healing and provides protection against the severity of upper respiratory infections, some procedure should be established to provide those submariners who smoke and who may restrict dietary intake of vitamin C with additional vitamin C supplementation.

This could be done by improving the availability or preparation of foods that are known to be high in vitamin C, or by encouraging daily intake of vitamin C supplements. After 400 years, the mistakes of the past should not be repeated.

FOOTNOTES

¹The opinions and interpretations contained in this article are those of the authors only, and do not represent the views, policies or endorsement of the U. S. Navy or any other government agency.

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TABLE I

Mean levels ($\pm$ SE) for plasma vitamin C (in mg/dl) during the pre-patrol, late patrol and post-patrol periods

Pre-patrol	Late-patrol	Post-patrol
1.23 ± 0.10	0.95 ± 0.12	1.40 ± 0.11