The relationship between antioxidants and immune function in healthy men

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Summary

Antioxidant vitamins and minerals have been linked to immune function, with deficiencies linked to an impaired immune function and supplementation linked to an improvement in immune function. However, few studies have either considered this relationship in healthy individuals in the community or accounted for potential confounders of the relationship, such as smoking, alcohol consumption, hepatitis and active exercise. This study explores, in 64 healthy men, the relationship between the blood concentrations of the antioxidants zinc, selenium, beta-carotene and vitamins A, C and E with immune function (as measured by the CD4 number), while taking into account the potential confounders, smoking, alcohol consumption, hepatitis and active exercise. Only beta-carotene showed a relationship with the CD4 number, a higher beta-carotene being associated with a lower CD4 number. As a lower CD4 number is a marker of reduced immune function, this indicates that higher blood levels of beta-carotene may be associated with a reduced immune function in these men.

Introduction

The antioxidant vitamins and minerals include the minerals zinc and selenium, and the vitamins A, E and C. Beta-carotene is often included, although it is not strictly a vitamin, because it both is a precursor for vitamin A and has antioxidant properties. There has been considerable interest in recent years in the effect that these vitamins and minerals may have on the immune function of individuals. Immune function can be measured in a variety of ways, most of them relying on the measurement of the amount or percentage of various immune-related cells in blood. One of the most commonly-used measures is that of the CD4 cell concentration, the so-called CD4 number. It is often used in HIV-infection as a measure of the strength of the immune system as the disease progresses. That is, as the disease progresses, the immune system weakens, resulting in a decreasing CD4 number.

The effect that various antioxidant minerals may have on the immune function have included reports that zinc deprivation causes immunodeficiency (1, 2) and that the severity of the disease in AIDS is related to a reduction in serum levels of zinc (3). Conversely, supplementation has been reported to improve immune function (2) and more specifically, the CD4 percentage (4). The mechanism may lie in zinc stimulating T-cell differentiation and maturation, stabilising cell membranes and increasing the number of circulating T-lymphocytes (3). Results such as these have resulted in calls for zinc supplementation in HIV-infection (3, 2). Similar results have been reported for selenium, with reduced blood concentrations of selenium associated with a reduction in immune function and the concentration of selenium being used as a marker of disease progression in HIV-infection (5).

There have been similar reports for antioxidant vitamins. For example, vitamin A deficiency has been linked to a reduction in the CD4 number and the CD4 to CD8 ratio (6, 7). Conversely, these are raised with vitamin A supplementation (7). The mechanism may lie with reports that CD4 cells are activated by vitamin A in vitro (8). Similar results have been reported for the precursor to vitamin A, beta-carotene (9). Consequently, supplementation of beta-carotene has been recommended in HIV-infection (9). Vitamin E has also been implicated in immune function. Once again, vitamin E deficiency has been linked to a reduction in immune function, this time in the aged (10), in rats (11) and in HIV-positive people (12, 13). Conversely, supplementation has

been linked to an improvement in immune function (10,12). The mechanism may lie in vitamin E playing an important role in the differentiation and maturation of T-cells in thymus. Consequently, calls for the supplementation of HIV-positive people with vitamin E have been made (12,13).

Reports such as this have become particularly important to those with diseases and syndromes that impair immune function, such as HIV/AIDS. Indeed, many people with HIV/AIDS routinely consume large quantities of these vitamins and minerals. This consumption has been promoted in literature that HIV-positive people often read, such the *Sydney Star Observer* newspaper and the *HIV Herald* journal.

However, of the work that has previously been done on the relationship between these vitamins and minerals and immune function, most investigators have considered only the simple relationship between vitamins and minerals on the one hand and immune function on the other. They have generally not considered the role of potential confounders of the relationship. Such potential confounders include the amount of smoking, the presence of hepatitis, alcohol consumption and the amount of active exercise. Smoking is included because it is known to have biochemically oxidising effects and may therefore have an effect on the concentration of antioxidants in the blood. Hepatitis and alcohol consumption are included because they are well-known reducers of immune function, to the extent that these are recognised reasons to immunise against many diseases. Active exercise is included because of the understanding amongst coaches of athletes that overtraining reduces immune function; the so-called 'overtraining syndrome'.

This study, therefore, explores the relationship between the concentration of these antioxidant vitamins and minerals and the CD4 number in 64 healthy men, whilst taking into account the previously-mentioned potential confounders.

Materials and Methods

Sixty four men, who considered themselves to be healthy, were enrolled. They were aged between 20 and 50 years. Because these data were also required for other analyses, comparing measurements between heterosexual and homosexual men, approximately half of the participants were homosexual. Consequently, in the resulting analyses of the data, sexuality was controlled-for and thus always appeared in the resultant multiple linear regression equations. All participants were tested and were found to be HIV-negative.

The health of participants was determined using a comprehensive questionnaire that sought information about past and present medical conditions, including hepatitis. Only a history of hepatitis proved to be of importance. A quarter of volunteers reported a history of hepatitis, most of these in homosexual men. Current hepatitis was measured as the concentration of liver function enzymes in the blood using routine methods at the Department of Clinical Chemistry at the Prince of Wales Hospital in Sydney. Work by others (14) and previous analyses on this data indicated that the most responsive and representative of these enzymes was alanine aminotransferase (ALT). Consequently, in these analyses, the concentration of ALT was used as a measure of hepatitis.

The CD4 number was measured by routine methods at the Department of Clinical Immunopathology at the Prince Henry Hospital. The concentration of the antioxidant vitamins and minerals zinc, selenium, vitamin A, vitamin E, vitamin C and beta-carotene in blood were measured by the Department of Clinical Chemistry at the Prince of Wales Hospital in Sydney.

Cigarette smoking was assessed in the questionnaire and expressed as the number of cigarettes smoked in the last month, because some individuals smoked irregularly or had recently changed their intake. Alcohol consumption was measured as the usual intake of a variety of alcoholic beverages using relevant questions in the Frequan Food Frequency Questionnaire and is reported here as grams of alcohol per day. Active exercise was measured via questionnaire as the number

of hours of exercise currently spent per week in 'active exercise that causes you to sweat and become breathless (eg jogging, swimming, squash, gym, aerobics)'.

The data were analysed using multiple linear regression.

Results

Participants in this study were found to have wide ranges of smoking and alcohol consumption, concentrations of ALT and amount of active exercise. They had a wide range of concentrations of antioxidant vitamins and minerals. Some took vitamin and mineral supplements. The CD4 number also varied widely, with several having low values. Consequently, the group of men was considered to be sufficiently heterogeneous to pick-up any relationship between the antioxidants measured and the CD4 number.

After adjusting for confounders, there was no significant relationship between the CD4 number and selenium (t=-1.06, P=0.30), zinc (t=-0.33, P=0.74), vitamin A (t = 1.4, P= 0.18), vitamin E (t = 0.73, P= 0.47) or vitamin C (t = 0.67, P= 0.51). The relationship between the CD4 number and beta-carotene, however was significant (t=-2.15, P=0.04). This indicated that, after adjusting for confounders, the CD4 number tended to rise significantly as the beta-carotene concentration fell.

Discussion and Conclusions

There are many relatively healthy people in society who consume antioxidant vitamins and minerals. Based on the reports of other investigators, many of these people are likely to be taking these substances in the belief that it will improve their immune function. The results above indicate that once confounders are taken into account, antioxidant vitamins and minerals appear to have little effect on the immune function of relatively healthy people, as assessed by the CD4 number. These results also question many of the links that have been made between these antioxidant vitamins and minerals and immune function in diseases such as HIV, because very few studies considered the confounding factors. As the major confounder here proved to be alcohol consumption, future studies should include the measurement of alcohol consumption.

The only antioxidant that appeared to have an effect on the CD4 number was beta-carotene, where the CD4 number tended to rise as the beta-carotene concentration fell. This indicates that a higher beta-carotene concentration may be associated with a reduced immune function. Such a result is counter to previous reports and should be further investigated in relatively healthy individuals. Further investigations should also measure markers of immune function in addition to the CD4 number, since the CD4 number, whilst being an important measure of immune function, is still only one of many measures. Further investigations should also take more than one measure of each antioxidant, as these levels can fluctuate over time.

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