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Nutrient intake and immune function of elderly subjects

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Abstract

Objective—Food intake, aging, and immune function share complex influences. Therefore, the purpose of this study was to examine relationships between nutrient intakes from food and dietary supplements and a biomarker of immune function.

Design—Data were collected from participants in a cross-sectional study as well as baseline data from a longitudinal study [n=89]. Subjects completed 24-hour food recalls including supplement intake [n=89]. Polyclonal mitogen phytohemmagluttin (PHA) was the immune function stimulator used. Height and weight were used to calculate body mass index.

Statistical analysis performed—Descriptive, bivariate correlation, Spearman's Rho for nonparametric data, t-tests, and stepwise regression with nutrient intakes as independent variables and T cell proliferation as dependent variables.

Results—Significant positive correlations (P≤ .05) were found between PHA-induced proliferation and intake of docosahexaenoic acid (DHA), eicosahexaenoic acid (EPA), sodium, and selenium, although intakes of DHA plus EPA were inadequate when compared to recommended intakes. A significant negative correlation with total vitamin A, with many vitamin A levels being above the upper limit of safety. Regression analyses found these nutrients to be variables significant in explaining the variance in PHA (p=0.005).

Conclusions—Selenium, sodium, DHA, EPA and vitamin A intake from diet and supplements were associated with PHA-induced proliferative responses. Clients may be counseled to have adequate selenium, EPA, DHA intake, and vitamin A, but avoid excess vitamin A.

The responsiveness of the human immune system has been shown to decrease with age (1). Generally the immune system is represented as either innate, which includes the complement system, natural killer cells, and phagocytosis, or adaptive. Adaptive immune include cell-mediated and humoral immunity. When innate immunity components are not able to clear the infection adaptive immunity components become involved. Both adaptive and innate immunity are adversely affected by aging in terms of the adaptiveness of T cells in the former and the effectiveness of natural killer cells in the latter (2). Specifically, T cell proliferation to

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polyclonal mitogens and specific pathogens is known to decrease with age (3). The clinical implications of immunosenescence include a poor response to vaccination, progressive loss in the ability to recognize foreign antigens, increased autoantibodies, and increased susceptibility to infections and chronic disease (4).

As a person ages, nutrient intake in general may be inadequate (5,6), increasing susceptibility for compromised immune function. A study comparing healthy elderly with healthy young adults showed that lower nutritional status was associated with the lower T cell functions in the elderly (7). While the effects of specific individual nutrient deficiencies on immune function are generally known, how aging, nutritional status, and immune function impact each other is not fully elucidated (8). This study examined nutrient intake, supplement use, and immune function and the relationships among these variables in an elderly community-living population.

METHODS

Subject Recruitment and Study Design

A convenience sample of males and females 65–80 years old were recruited from the Champaign, Illinois vicinity through local media and contacts with area health facilities, senior centers, and additional public places. This study used data from two sources: a cross-sectional study and the baseline data from longitudinal study of aging and immune function and exercise intervention. Only independently community living individuals were recruited. Subjects responding to the recruitment campaign were pre-screened by telephone and sent eligibility questionnaires. Subjects were selected based on age, gender, and medication usage. Exclusion criteria included a self-reported history of: cancer or strong allergies, abnormal blood tests (complete blood counts, fasting glucose, potassium), primary immunodeficiencies, mental illness, pre-menopausal status, recent (< 6 months) vaccination, illness, smoking, alcohol or drug abuse, heart disease, involvement in competitive athletics or a highly physically active lifestyle. All participants signed informed consent according to the approved University of Illinois Institutional Review Board protocol. Attempts were made to obtain a full vaccination history from each participant, including whether or not they received a full primary tetanus series. Subjects who had a tetanus booster within the previous 3 years were excluded. Blood was drawn and transferred to the medical center laboratory on ice as part of routine blood testing for complete blood counts, comprehensive metabolic panel, and total cholesterol. Participants would have been excluded if results were outside of the normal range, but all were normal (Normal ranges in Table 1).

All subjects were asked to refrain from use of certain medications such as corticosteroids, non-steroidal anti-inflammatory drugs, or anti-histamines 4 days prior to immune testing.

Diet Assessment

Subjects were instructed on recording their intakes from the previous day relative to portion sizes and specificity of foods eaten, and asked to complete a 1-day food recall at the lab. A registered dietitian reviewed the recall with each participant, clarifying food terms, ingredients, brands, and portion sizes, following a method similar to the multi-pass method so that the 1-day food recall was reviewed multiple times for portions, forgotten foods, and clarification of any food items (9,10). Information about supplement use was obtained at the time of 1-day food recall review as well, including brand and quantity for supplement taken. Subjects whose recall represented a non-typical day were omitted from the analysis (n=4). Dietary Reference Intakes (DRI) and their Estimated Average Requirement (EAR), Recommended Dietary Allowances (RDA) or Adequate Intake (AI) values were used to assess nutrient intake adequacy (11–17), the exception being docosahexaenoic acid (DHA) and eicosahexaenoic acid

(EPA). The range of optimal intake of 0.5 to 1.8 g/day for combination of DHA and EPA from diet and supplements and intake of α-linolenic acid of 1.5 to 3.0 g/day were used for reference ranges (18).

Anthropometrics

Height was measured without shoes using a stadiometer to the nearest 0.25 inch (Seca,Corp., Hamburg, Germany). Weight was measured on a calibrated digital scale to the nearest 0.25 pound (Tanita BWB-600, Wedderburn, Australia). An average of three measures was used to ensure accuracy. Body mass index (BMI) was calculated as kg/meter^2 and compared to the classification of BMI of the National Heart, Lung, and Blood Institute (19).

Serum Measures

Fasting blood (12 hours) was drawn for immune function, transferred on ice to the medical center laboratory for i*n vitro* proliferation after stimulation with the polyclonal mitogen phytohemagglutinin (PHA) determinations. Healthy lymphocytes respond to PHA stimulation by proliferating vigorously in a polyclonal manner. The ability to proliferate in such a way has been routinely used as a general indicator of T lymphocyte function (20). Triplicate cultures were stimulated for 3 days with PHA in concentration of 0.5, 1.0, 2.0, 4.0, and 8.0 μg/ml (Sigma, St. Louis, MO) in 50 μl volumes in 96-well round bottom plates. All cultures were maintained in AIM-V media and incubated at 37°C in 5% CO_2. After 3 day culture, 1.0 μCi of (methyl-^{3}H) thymidine (ICN, Irvine, CA) was added to each cell culture well. The amount of radioactivity incorporated into the cultures was determined by harvesting the contents of each well after 4 hours incubation with tracer onto glass fiber filters using a cell harvester (Cambridge Technologies, Watertown, MA). Radioactivity of the filters was determined using a Packard liquid scintillation counter (Packard, Meridian, CT). Net proliferation was calculated as stimulant cultures (PHA) minus cultures containing no stimulant. The same lot numbers of PHA were utilized in all experiments.

Data Analyses

Twenty-four hour recalls were analyzed using Nutritionist V (First Data Bank, version 2.3, San Bruno, CA, 2000) and entered into SPSS for analyses. Quality assurance for data entry included outlier examination using boxplots and verification with original data source as well as continuous random inspection data entry and original source equivalency. Statistical analysis for descriptive, bivariate correlation, Spearman's Rho for nonparametric data, t-tests, and stepwise regression with nutrient intakes as independent variables and t cell proliferation as dependent variables were completed using SPSS with significance set at ($P < .05$) (version 15.0, Chicago, IL, 2006).

RESULTS

Thirty-six men and 57 women participated and had complete dietary, supplement, and PHA data. Four were omitted from analyses because they had non-typical food intake during the recall period, leaving 35 men and 54 women for analyses. Their mean age was 69.7 ± 5.5 years and a mean BMI of 27.8 ± 4.7 kg/m^2 (Table 1). Means did not differ by gender. Only 20 (22%) of participants were classified as normal weight, with most being overweight or obese, and 3 classified as extremely obese.

Mean nutrient intakes for diet alone and diet plus supplements are listed in Table 2. Mean energy intake for women was 1638 ± 458 kcal and for men 2177 ± 596. Most (80%) achieved recommended caloric and protein intake (78%). Many consumed a diet higher than recommended in total fat (≥ 35% calories by 60% of participants) and saturated fat (>10% calories by 49% of participants). Few participants met recommended levels for linoleic and

linolenic acid (≤ 35 % of participants), and only 6% had diets meeting the DHA plus EPA recommendations. More than 50% of participants had diets not meeting the recommended amounts for potassium, calcium, magnesium, vitamin D, vitamin E, vitamin K, folate, and pantothenic acid (Table 3).

Supplements improved nutrient intake for all nutrients included in the supplements ($P < .05$). With supplementation to the diet, there was an increase in the percentage of subjects meeting the recommended levels for all vitamins and minerals. However, vitamin K intake did not improve greatly for most despite the dietary supplementation. In addition, >25% met or exceeded the upper limit of safety for niacin, magnesium and vitamin A (Table 3).

Bivariate correlations were used to assess the relationship between T cell PHA-induced proliferation and nutrient intake, with Spearman's Rho when data were nonparametric as assessed by Kolmogorov-Smirnov one-sample test. Poor proliferative response being associated with less immunocompetence and greater prolierative response with better immunocompetence. Significant correlations were found between PHA proliferation (0.5, 1.0, and 2.0 μg/ml) and DHA plus EPA intake, and for DHA or EPA alone (0.5, 1.0, and 2.0 μg/ml). Significant negative correlations were found for total vitamin A (2.0, 4.0, and 8.0 μg/ml) and positive correlations for dietary selenium and sodium intake at higher concentrations of PHA (2.0, 4.0, and 8.0 μg/ml) (Table 4).

Stepwise regression analysis was used to determine if nutrient intakes as independent variables were able to predict the variation in PHA-induced proliferation. Five significant equations were found, explaining 9 to 58% of the variance (Table 5). Significant variables in these equations included DHA plus EPA, fat, dietary selenium, and vitamin A.

DISCUSSION

Although zinc deficiency has been associated with impaired immune function, zinc intake was adequate for most subjects with diet alone, similar to a European study of community-dwelling elders (21). Total zinc intake (supplemental zinc plus dietary zinc) was adequate for all but 9 men and 4 women. Similarly, intakes from diet alone were in the range of those found for elderly European men and women for vitamin A and folate (22). Comparison with US studies of dietary intake of the elderly are difficult since results are often reported as percentages of recommended intake levels that are no longer current (23) or had recruitment criteria that included certain dietary patterns (24).

The deficiency of a number of nutrients have been linked to immune function, notably vitamin A (25), vitamin C and zinc (26), vitamin B_6 (27), iron and copper (28) and selenium (29). Few of the participants in the current study had dietary intakes of these nutrients below the recommended levels, and most of those reached the recommended level with supplementation, an exception being selenium where 17 individuals continued to not meet recommended selenium intakes.

Marginal selenium status has been associated with compromised immune function (30). Selenium supplementation in animals has been shown to improve immune function. For lymphocytes, improvement in proliferation was reported if the supplementation was low, but was inhibited if supplementation was high (31). In humans, selenium supplementation has also been reported to have a beneficial effect on immune function (32,33). Results of the present study could not suggest selenium deficiency, but is in accordance with an association between selenium intake and immune function. Mean dietary selenium was improved significantly with supplements, but 31 participants had no supplement containing selenium, 51 had low levels of supplementation (20 μg) and 6 had supplements of 70–120 μg. A recent study reported a

correlation between blood selenium levels and immune function with an increased proliferation in lymphocytes that reflects the findings of the present study (34).

The omega-3 fatty acids DHA and EPA were significantly correlated with lymphocyte proliferation at the 0.5, 1.0, and 2.0 μg/ml concentrations and were a significant variable in regression equations explaining the variance in proliferation at these concentrations as well. However, these relationships were not found at higher lymphocyte concentrations. Clinical trials of relative high levels of DHA have demonstrated no effect on lymphocyte proliferation (35–37). Although EPA and DHA have been reported to suppress lymphocyte proliferation (38,39) these *in vitro* studies added EPA to cell cultures creating a much different interaction than dietary EPA may have *in vivo*. A small study of 4 volunteers consuming 2.4 g EPA/day for 6 weeks reported no consistent changes in immune responses (40). Forty-two participants received either placebo, 4.7 g EPA, or 4.9 g DHA for 4 weeks. Lymphocyte activation as measured by CD69 expression, an early marker of lymphocyte proliferation, was suppressed by DHA. This is in contradiction to others reports and may reflect an affect on function but not proliferation (41).

Total vitamin A intake included dietary and supplemental vitamin A but did not include dietary or supplemental β carotene. Total vitamin A intake was negatively correlated with lymphocyte proliferation at 2.0, 4.0, and 8.0 μg/ml and was a negative variable in significant regression equations for these levels of PHA. While deficiency of vitamin A has been associated with a negative effect on immune function (25), our data do not reflect poor vitamin A intake. Supplementation resulted in intakes at or above the UL for many participants. Vitamin A supplementation has been reported to have a negative immune function effect in the elderly (42), and could account for the negative correlation and negative weight in regression equations in the current study.

The relationship between sodium intake and immune response was not expected. Sodium intake has been associated with hypertension in population-based observational studies as well as intervention studies (17). Interestingly, there is growing evidence indicating a relationship between immune function and hypertension. Not only may vascular damage elicit immune responses, but also that an abnormal immune system may contribute to the develop of hypertension (43,44). The current correlation supports neither of these hypotheses but does suggest that additional research may be warranted.

While relationships between nutrition and immunology in the elderly have been identified both in the present study and previous research, the relationships are not distinct nor are mechanisms of interaction known. Although protein energy malnutrition has a detrimental effect on immunity (45), none of these participants were underweight. Conversely overweight and obesity have been shown to be associated with increased immune cell counts in women, although PHA were not specifically assessed (46). The number of participants in each BMI category was too small to adequately assess the impact of under- or overnutrition on PHA.

There are several limitations inherent in any study of dietary intake. Using the EAR for assessing dietary adequacy is complex but can be used to examine the probability that intake is inadequate for individuals or to assess the prevalence of inadequate intakes within a group (14). However, EAR have only been calculated for the B vitamins, vitamins C and E, selenium, and phosphorus and RDA should not be used for assessing group data. Although 24-hour recalls are more reliable than food frequency questionnaires (47), they are still subjective tools that represent a one day’s frame of usual intake. As with any dietary analysis, the completeness of the database may under-represent nutrient intake. An earlier version of the database used compared favorably with other databases analyzed (48), however food products continue to change and manufacturer's analysis may not include EPA and DHA in particular.

In addition, this is a cross-sectional study and not a nutrition intervention project. A previous small intervention with community-living elderly [n=31] found no effect of a micronutrient supplement on lymphocyte proliferation as measured by concanavalin A (49). Authors concluded that adequate intake of nutrients known to affect immune health was a message for all elders. Our study concurs with this conclusion but adds that dietitians should assess for high as well as low nutrient intake.

CONCLUSIONS

Selenium, omega-three fatty acids, sodium and vitamin A intake from diet and supplements were found to be associated with *in vitro* T lymphocyte PHA-induced proliferative responses. Lower than recommended intakes of selenium and omega-three fatty acids and higher than recommended intakes of vitamin A should be further investigated for their impact on overall immune function in the elderly. Dietitians need to assess dietary and supplemental vitamin A in relation to the UL to prevent excess vitamin A intake in their elderly clients. Clients should be counseled concerning good dietary sources of EPA and DHA as well as selenium to improve or maintain dietary status.

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References

1. Chin, A Paw; De Jong, N.; Pallast, E.; Kloek, G.; Schouten, E.; Kok, F. Immunity in frail elderly: a randomized controlled trial of exercise and enriched foods. J Amer Coll Sports Med 2000:2005–2011.
2. Graham JE, Christian LM, Kiecolt-Glaser JK. Stress, age, and immune function: Toward a lifespan approach. J Behav Med 2006;29(4):389–400. [PubMed: 16715331]
3. Nagel J, Chopra R, Chrest F, McCoy M, Schneider E, Holbrook, Alder W. Decreased proliferation, interleukin 2 synthesis, and interleukin 2 receptor expression are accompanied by decreased mRNA expression, in phytohemagglutinin-stimulated cells from elderly donors. J Clin Invest 1988;81:1096–1102. [PubMed: 3127423]
4. Prelog M. Aging of the immune system: a risk factor for autoimmunity? Autoimmun Rev 2006;5(2):136–139. [PubMed: 16431345]
5. Vaquero MP. Magnesium and trace elements in the elderly: intake, status and recommendations. J Nutr Health Aging 2002;6(2):147–153. [PubMed: 12166371]
6. Ritchie CS, McClave SA. Part II. Common nutritional issues in older adults. Dis Mon 2002;48(11):713–724. [PubMed: 12474014]
7. Mazari L, Lesourd B. Nutritional influences on immune response in healthy ages persons. Mechanisms Ageing Devel 1998;104:25–40.
8. Wintergerst ES, Maggini S, Hornig DH, Wintergerst ES. Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metab 2007;51(4):301–323. [PubMed: 17726308]
9. Raper N, Perloff B, Ingwersen L, Steinfeldt L, Anand J. An overview of USDA's dietary intake data system. J Food Compos Anal 2004;17:545–555.
10. Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and non-obese women. Amer J Clin Nutr 2003;77:1171–1178. [PubMed: 12716668]
11. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride. Washington, DC: National Academy Press; 1997.
12. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B_6, Folate, vitamin B_{12}, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press; 1998.
13. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids. Washington, DC: National Academy Press; 2000a.

14. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes: Applications in Dietary Assessment. Washington, DC: National Academy Press; 2000b.
15. Institute of Medicine, Food and Nutrition Boar. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. Washington, DC: National Academy Press; 2001.
16. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acid, Cholesterol, Protein, and Amino Acids. Washington, DC: National Academy Press; 2002.
17. Institute of Medicine, Food and Nutrition Board. Dietary Reference Intakes for Water, Potassium, Sodium, Chloride, and Sulfate. Washington, DC: National Academy Press; 2005.
18. Kris-Etherton P, Harris WS, Appel LJ. American Heart Association. Nutrition Committee: Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation 2002;106(21): 2747–2757. [PubMed: 12438303]
19. National Heart Lung and Blood Institute, US Department of Health and Human Services. Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults: The Evidence Report. 1998NIH Publication 98-4083
20. Whiteside, TL. Antigen/mitogen-stimulated proliferation. In: Lotze, MT.; Thomson, AW., editors. Measuring Immunity: Basic Biology and Clinical Assessment. London: Elsevier; 2005. p. 361-368.
21. Andriollo-Sanchez M, Hininger-Favier I, Meunier N, Toti E, Zaccaria M, Brandolini-Bunlon M, Polito A, O'Connor JM, Ferry M, Coudray C, Roussel AM. Zinc intake and status in middle-aged and older European subjects: the ZENITH study. Eur J Clin Nutr 2005;59:S37–S41. [PubMed: 16254579]
22. Polito A, Intorre F, Andriollo-Sanchez M, Azzini E, Raguzzini A, Meunier N, Ducros V, O'Connor JM, Coudray C, Roussel AM, Maiani G. Estimation of intake and status of vitamin A, vitamin E and folate in older European adults: the ZENITH. Eur J Clin Nutr 2005;59:S42–S47. [PubMed: 16254580]
23. Kant AK, Schatzkin A. Relation of age and self-reported chronic medical condition status with dietary nutrient intake in the US population. J Am Coll Nutr 1999;18(1):69–76. [PubMed: 10067661]
24. Barr SI, McCarron DA, Heaney RP, Dawson-Hughes B, Berga SL, Stern JS, Oparil S. Effects of increased consumption of fluid milk on energy and nutrient intake, body weight, and cardiovascular risk factors in healthy older adults. J Am Diet Assoc 2000;100(7):810–817. [PubMed: 10916520]
25. Villamor E, Fawzi WW. Effects of vitamin a supplementation on immune responses and correlation with clinical outcomes. Clin Microbiol Rev 2005;18(3):446–464. [PubMed: 16020684]
26. Wintergerst ES, Maggini S, Hornig DH. Immune-enhancing role of vitamin C and zinc and effect on clinical conditions. Ann Nutr Metab 2006;50(2):85–94. [PubMed: 16373990]
27. Trakatellis A, Dimitriadou A, Trakatelli M. Pyridoxine deficiency: new approaches in immunosuppression and chemotherapy. Postgrad Med J 1997;73(864):617–622. [PubMed: 9497969]
28. Arredondo M, Nunez MT. Iron and copper metabolism. Mol Aspects Med 2005;26(4–5):313–327. [PubMed: 16112186]
29. Cunningham-Rundles S, McNeeley DF, Moon A. Mechanisms of nutrient modulation of the immune response. J Allergy Clin Immunol 2005;115(6):1119–1128. [PubMed: 15940121]
30. Rayman MP, Rayman MP. The argument for increasing selenium intake. Proc Nutr Soc 2002;61(2): 203–215. [PubMed: 12133202]
31. Finch JM, Turner RJ. Effects of selenium and vitamin E on the immune responses of domestic animals. Res Vet Sci 1996;60:97–106. [PubMed: 8685547]
32. Roy M, Kiremidjian-Schumacher L, Wishe M, Cohen W, Stotzky G. Supplementation with selenium and human immune cell functions. I. Effect on lymphocyte proliferation and interleukin 2 receptor expression. Biol Trace Elem Res 1994;41:103–114. [PubMed: 7946898]
33. Kiremidjian-Schumacher L, Roy M. Effect of selenium on the immunocompetence of patients with head and neck cancer and on adoptive immunotherapy of early and established lesions. Biofactors 2001;14(1–4):161–168. [PubMed: 11568453]
34. Dunstan JA, Breckler L, Hale J, Lehmann H, Franklin P, Lyons G, Ching SY, Mori TA, Barden A, Prescott SL. Associations between antioxidant status, markers of oxidative stress and immune

responses in allergic adults. Clin Exp Allergy 2006;36(8):993–1000. [PubMed: 16911355]Erratum in: *Clin Exp Allergy*. 2006;36(11):1480

35. Kelley DS, Taylor PC, Nelson GJ, Mackey BE. Dietary docosahexaenoic acid and immuncompetence in young healthy men. Lipids 1998;33:559–566. [PubMed: 9655370]
36. Kelley DS, Taylor PC, Nelson GJ, Schmidt PC, Ferretti A, Erickson K, Yu R, Chandra RK, Mackey BE. Docosahexaenoic acid ingestion inhibits natural killer cell activity and production of inflammatory mediators in young healthy men. Lipids 1999;34:317–324. [PubMed: 10443964]
37. Thies F, Nebe-von-Caron G, Powell JR, Yaqoob P, Newsholme EA, Calder PC. Dietary supplementation with gamma-linolenic acid or fish oil decreases T lymphocyte proliferation in healthy older humans. J Nutr 2001;131(7):1918–1927. [PubMed: 11435508]
38. Khalfoun B, Thibault G, Lacord M, Gruel Y, Bardos P, Lebranchu Y. Docosahexaenoic and eicosapentaenoic acids inhibit human lymphoproliferative responses in vitro but not the expression of T cell surface activation markers. Scand J Immunol 1996;43(3):248–256. [PubMed: 8602457]
39. Terada S, Takizawa M, Yamamoto S, Ezaki O, Itakura H, Akagawa KS. Suppressive mechanisms of EPA on human T cell proliferation. Microbiol Immunol 2001;45(6):473–481. [PubMed: 11497223]
40. Virella G, Fourspring K, Hyman B, Haskill-Stroud R, Long L, Virella I, La Via M, Gross AJ, Lopes-Virella M. Immunosuppressive effects of fish oil in normal human volunteers: correlation with the in vitro effects of eicosapentanoic acid on human lymphocytes. Clin Immunol Immunopathol 1991;61 (2 Pt 1):161–176. [PubMed: 1833105]
41. Kew S, Mesa MD, Tricon S, Buckley R, Minihane AM, Yaqoob P. Effects of oils rich in eicosapentaenoic and docosahexaenoic acids on immune cell composition and function in healthy humans. Am J Clin Nutr 2004;79(4):674–681. [PubMed: 15051614]
42. Fortes C, Forastiere F, Agabiti N, Fano V, Pacifici R, Virgili F, Piras G, Guidi L, Bartoloni C, Tricerri A, Zuccaro P, Ebrahim S, Perucci CA. The effect of zinc and vitamin A supplementation on immune response in an older population. J Am Geriatr Soc 1998;46(1):19–26. [PubMed: 9434661]
43. Fu ML. Do immune system changes have a role in hypertension? J Hypertens 1995;13(11):1259–1265. [PubMed: 8984123]
44. Buemi M, Marino D, Floccari F, Ruello A, Nostro L, Aloisi C, Marino MT, Di Pasquale G, Corica F, Frisina N. Effect of interleukin 8 and ICAM-1 on calcium-dependent outflow of K+ in erythrocytes from subjects with essential hypertension. Curr Med Res Opin 2004;20(1):19–24. [PubMed: 14741067]
45. Schaible UE, Kaufmann SH. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 2007;4(5):e115. [PubMed: 17472433]
46. Womack J, Tien PC, Feldman J, Shin JH, Fennie K, Anastos K, Cohen MH, Bacon MC, Minkoff H. Obesity and immune cell counts in women. Metabolism 2007;56(7):998–1004. [PubMed: 17570264]
47. Buzzard IM, Faucett CL, Jeffery RW, McBane L, McGovern P, Baxter JS, Shapiro AC, Blackburn GL, Chlebowski RT, Elashoff RM, Wynder EL. Monitoring dietary change in a low-fat diet intervention study: advantages of using 24-hour dietary recalls vs food records. J Am Diet Assoc 1996;96(6):574–579. [PubMed: 8655904]
48. McCullough ML, Karanja NM, Lin PH, Obarzanek E, Phillips KM, Laws RL, Vollmer WM, O'Connor EA, Champagne CM, Windhauser MM. DASH Collaborative Research Group. Comparison of 4 nutrient databases with chemical composition data from the Dietary Approaches to Stop Hypertension trial. J Am Diet Assoc 1999;99:S45–S53. [PubMed: 10450294]
49. Boardley D, Fahlman M. Micronutrient Supplementation does not Attenuate Seasonal decline of immune system indexes in well-nourished elderly women: A placebo-controlled study. J Am Diet Assoc 2000;100(3):356–359. [PubMed: 10719412]

Table 1

Normal lab values

Test	Expected Range	Units
Hematology:		
White Blood Cells	4.00–11.00	10^3/ul
Red Blood Cells	4.10–5.70	10^6/ul
Hemoglobin	12.0–18.0	g/dl
Hematocrit	37.0–51.0	%
Mean Corpuscular Volume	80.0–100.0	fl
Mean Corpuscular Hemoglobin	27.0–33.0	pg
Mean Corpuscular Hemoglobin Concentration	32.0–36.0	g/dl
Red Blood Cell Distribution Width	12.0–15.0	%
Red Blood Cell Distribution Width Standard Deviation	38.0–52.0	fl
Platelet	140–400	10^3/ul
Mean Platelet Volume	9.0–12.0	fl
Neutrophil	40.0–70.0	%
Lymphocyte	20.0–45.0	%
Monocyte	0.0–10.0	%
Eosinophil	0.0–5.0	%
Basophil	0.0–2.0	%
Absolute Neutrophil	1.60–7.70	10^3/ul
Absolute Lymphocyte	1.00–4.90	10^3/ul
Absolute Monocyte	0.00–1.10	10^3/ul
Absolute Eosinophil	0.00–0.50	10^3/ul
Absolute Basophil	0.00–0.20	10^3/ul
Chemistry:		
Calcium	8.5–10.5	mg/dl
Glucose	60–99	mg/dl
Blood Urea Nitrogen	6–20	mg/dl
Creatinine	0.5–1.2	mg/dl
Protein, Serum Total	6.0–8.1	gm/dl
Albumin	3.2–5.5	gm/dl
Bilirubin, Total	0.2–1.0	mg/dl
Aspartate Aminotransferase	12–50	IU/L
Alanine Aminotransferase	10–75	IU/L
Alkaline Phosphatase	42–121	IU/L
Sodium	135–145	mmol/L
Potassium	3.6–5.0	mmol/L
Chloride	101–111	mmol/L
Carbon Dioxide	21.0–31.0	mmol/L
Cholesterol, Total	<200	mg/dl

Table 2

Nutritional adequacy of diet compared to nutritional adequacy of diet with supplementation in elderly subjects, n=89

Nutrient	EAR or AI for age group	% reaching EAR or AI with diet alone (n)	% reaching EAR or AI with diet + supp. (n)	% ≥ UL, diet + supp. (n)
Calories[16]				
Males	2,587	20 (7)	20 (7)	
Females	2,067	20 (11)	20 (11)	
Protein g/kg[16]	0.66	78 (69)	78 (69)	
Carbohydrate g[16]	100	96 (85)	96 (85)	
Fat[16]	35 % total energy	60 (53)	60 (53)	
Saturated fat[16]	10 % total energy	49 (44)	49 (44)	
Monounsaturated fat[16]	15 % total energy	89 (79)	89 (79)	
Polyunsaturated fat[16]	10 % total energy	89 (79)	89 (79)	
Linoleic acid g[16]				
Males	14	9 (3)	9 (3)	
Females	11	22 (12)	22 (12)	
Linolenic acid g[16]				
Males	1.6	9 (3)	9 (3)	
Females	1.1	24 (13)	24 (13)	
DHA + EPA[18]	0.3–0.5	6 (5)	13 (12)	
Sodium g[17]	1.2	89 (100)	89 (100)	
Potassium g[17]	4.7	6 (5)	6 (5)	
Vitamin A (mcg RE)[15]				
Males	624	89 (31)	91 (32)	29 (10)
Females	500	93 (50)	93 (50)	36 (23)
Vitamin C mg[13]				
Males	75	71 (25)	89 (31)	
Females	60	93 (50)	98 (53)	
Vitamin D IU[11]	400	4 (4)	69 (61)	1
Vitamin E mg[13]	12	13 (12)	74 (66)	1 (supp. only)
Vitamin K mcg[15]				
Males	120	14 (5)	14 (5)	
Females	90	31 (17)	31 (17)	
Thiamin mg[12]				
Males	1.0	83 (29)	91 (32)	
Females	0.9	87 (47)	96 (52)	
Riboflavin mg[12]				
Males	1.1	89 (31)	94 (33)	
Females	0.9	87 (47)	96 (52)	
Niacin mg[12]				
Males	12	89 (31)	94 (33)	59 (20)
Females	11	80 (43)	91 (49)	54 (28)

Nutrient	EAR or AI for age group	% reaching EAR or AI with diet alone (n)	% reaching EAR or AI with diet + supp. (n)	% ≥ UL, diet + supp. (n)
Vitamin B-6 mg[12]				
Males	1.4	63 (22)	80 (28)	
Females	1.3	69 (37)	87 (47)	
Folate mcg DFE[12]	544	38 (34)	74 (66)	6 (5)
Vitamin B-12 mcg[12]	2.0	71 (63)	90 (80)	
Pantothenic acid mg[12]	5	13 (12)	66 (59)	
Calcium mg[11]	1200	19 (17)	42 (37)	3 (3)
Iron mg[15]	8	91 (81)	92 (82)	3 (3)
Magnesium mg[11]				
Males	350	20 (7)	46 (16)	49 (17)
Females	265	43 (23)	72 (39)	39 (21)
Zinc mg[15]				
Males	9.4	40 (14)	74 (26)	3 (1)
Females	6.8	72 (39)	93 (50)	4 (2)
Selenium mcg[13]	45	64 (57)	81 (72)	

EAR=estimated average requirements

AI=adequate intake

UL=upper level of safety

RE= Retinyl equivalents

EPA = eicosahexaenoic acid

DHA = docosahexaenoic acid

DFE= dietary folate equivalents

Table 3

Mean (standard deviation) intakes of nutrients from diet alone and diet plus supplements in elderly subjects, n=89

	Mean ± SD Diet only	Mean ± SD Diet + supplements	t,p^1
Potassium, mg	2814.6 ± 1011.8	2864.1 ± 1022.7	11.9, .0001
Vitamin A, mcg RE	1225.1 ± 933.4	2098.6 ± 3225.9	2.6, .010
β-carotene, mcg RE	1232.2 ± 3477.4	1727.7 ± 3463.3	13.5, .0001
Vitamin C, mg	165.5 ± 187.8	315.7 ± 295.5	5.7, .0001
Vitamin D, IU	190.3 ± 494.4	498.2 ± 498.0	12.8, .0001
Vitamin E, IU	9.9 ± 9.0	153.7 ± 212.5	6.4, .0001
Vitamin K ug	79.9 ± 100.7	86.5 ± 100.9	12.8, .0001
Thiamin, mg	1.6 ± 0.8	2.6 ± 1.1	13.2, .0001
Riboflavin, mg	1.7 ± 0.8	2.9 ± 1.2	13.2, .0001
Niacin, mg	19.4 ± 9.1	32.6 ± 13.0	13.2, .0001
Vitamin B_6 mg	1.8 ± 0.8	5.3 ± 9.3	3.6, .0001
Folate, ug DFE	518.4 ± 272.2	953.9 ± 454.9	11.9, .0001
Vitamin B_{12} ug	3.8 ± 3.2	28.0 ± 61.7	3.7, .0001
Pantothanic acid, ug	3.6 ± 2.8	9.9 ± 5.7	12.2, .0001
Calcium, mg	790.2 ± 449.7	1135.9 ± 609.3	8.1, .0001
Iron, mg	14.8 ± 6.6	18.4 ± 9.3	4.7, .0001
Magnesium, mg	286.0 ± 112.3	351.2 ± 127.7	12.8, .0001
Zinc, mg	9.9 ± 5.3	21.4 ± 13.0	8.9, .0001
Selenium, mg	0.1 ± 0.04	17.9 ± 22.0	7.7, .0001

1 paired t, significance

RE=retinol equivalents

β-carotene reported in IU, converted from μg by conversion factor of 3.33

IU=international units

DFE= dietary folate equivalents

Table 4

Correlation between polyclonal mitogen response [PHA] with nutrients in elderly subjects, n=89

	EPA + DHA	Protein g/kg	DHA (diet)	Vitamin A RE (diet)	Vitamin A (total)	Sodium (diet)	EPA (diet)	Riboflavin (diet)	Selenium (diet)
	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)
PHA 0 μg/ml	.09 (.387)	.10 (.341)	.09 (.405)	−.04 (.694)	−.18 (.103)	.12 (.258)	.10 (.346)	−.05 (.670)	.06 (.606)
PHA .5 μg/ml	**.65 (<.001)**	−.12 (.262)	**.67 (<.001)**	−.07 (.515)	−.16 (.158)	.09 (.409)	**.59 (<.001)**	**.23 (.034)**	.11 (.296)
PHA 1 μg/ml	**.52 (<.001)**	−.07 (.491)	**.53 (<.001)**	−.07 (.501)	−.10 (.355)	.08 (.417)	**.48 (<.001))**	**.23 (.033)**	.18 (.094)
PHA 2 μg/ml	**.26 (.015)**	**−.21 (.046)**	**.27 (.012)**	−.17 (.113)	**−.23 (.037)**	**.21 (.046)**	**.23 (.029)**	.13 (.220)	**.29 (.006)**
PHA 4 μg/ml	.02 (.839)	−.19 (.069)	.03 (.810)	**−.21 (.044)**	**−.26 (.018)**	**.25 (.020)**	.01 (.928)	.06 (.607)	**.27 (.009)**
PHA 8 μg/ml	−.07 (.545)	−.15 (.155)	−.06 (.572)	−.20 (.063)	**−.26 (.016)**	**.22 (.043)**	−.08 (.479)	−.02 (.878)	**−.12 (.264)**

EPA = eicosahexaenoic acid

DHA = docosahexaenoic acid

Values bolded are significant $p \leq 0.05$.

Table 5

Regression analyses explaining variance in immune function response, n=89

Dependent Variable	Independent variables	F	P
T-cell proliferation with PHA concentration of .5 μg/ml	8296 + 113969 [DHA diet] – 72749 [EPA + DHA diet] + 4 [fat] - .14 [vitamin A total]; R = .763; R^2 = .582	27	.028
T-cell proliferation with PHA concentration of 1.0 μg/ml	12183 + 20921 [DHA diet] + 20 [saturated fat kcal]; R = .566; R^2 = .320	20	.038
T-cell proliferation with PHA concentration of 2.0 μg/ml	33246 + 170124 [selenium] – 11761 [protein g/kg] + 11638 [DHA]; R = .475, R^2 = .225	8	.043
T-cell proliferation with PHA concentration of 4.0 μg/ml	54593 + 230093 [selenium] – 18099 [protein g/kg]; R = .368; R^2 = .135	7	.016
T-cell proliferation with PHA concentration of 8.0 μg/ml	48195 + 84 [carbohydrate] – 8 [vitamin A RE diet]; R = .307; R^2 = .094	4	.038

PHA= Polyclonal mitogen phytohemmagluttin

EPA = Eicosahexaenoic acid

DHA = Docosahexaenoic acid

Vitamin A RE = Retinol equivalents