Lead Article

Update: Effects of Antioxidant and Non-Antioxidant Vitamin Supplementation on Immune Function

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The purpose of this manuscript is to review the impact of supplementation with vitamins E and C, carotenoids, and the B vitamins on parameters of innate and adaptive immune function as reported from clinical trials in humans. There is evidence to support causal effects of supplementation with vitamins E and C and the carotenoids singly and in combination on selected aspects of immunity, including the functional capacity of innate immune cells, lymphocyte proliferation, and the delayed-type hypersensitivity (DTH) response. Controlled intervention trials of B vitamin-containing multivitamin supplements suggest beneficial effects on immune parameters and clinical outcomes in HIV-positive individuals.

INTRODUCTION

The synergistic and interactive relationship between nutrition and infection, and the importance of this for human health was summarized in a pivotal review by Scrimshaw in 1968.[1] That review provided ample support for the concept that general malnutrition, manifested as deficiencies in either multiple or specific nutrients, increases susceptibility to and severity of infections, likely via modulation of immune function.[1] The conclu-

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Editor's Note: This manuscript contains four very large tables. Table 1 is printed here, and the other three are available on the publisher's website at: www. ilsi.org/Publications/NutritionReviews/.

sions reached in this early monograph energized research on the impact of vitamin status on immune function and the risk of infectious diseases. Previous reviews of the literature have focused primarily on findings from cell and animal studies or observational studies in deficient human populations.[2-5] The purpose of the current review is the impact of supplementation with vitamins E and C, the carotenoids, and the B vitamins on parameters of immune function as reported from clinical trials in humans. The influence of preformed vitamin A on immune function and clinical outcomes has been previously reviewed.[6]

For each vitamin or combination of vitamins, we will first review findings from clinical trials that investigated the impact of supplementation on parameters of innate immunity, including cell numbers and functions and soluble mediators. Next we will review the effects of supplementation on parameters of adaptive immunity, including lymphocyte counts, proliferation, and cytokine production, measures of cell-mediated immunity, including the delayed type hypersensitivity response (DTH), and measures of humoral immunity, including T-cell-dependent and T-cell-independent antibody production. While non-controlled supplementation studies are considered, emphasis is placed on findings from randomized, controlled clinical trials (RCTs) and tables that summarize these findings are provided for each vitamin or their combinations. Finally, we discuss how the potential effects of vitamin supplementation on immune parameters correlate with findings from RCTs that have examined the impact of supplementation on clinical outcomes related to infectious diseases.

SUPPLEMENTATION WITH VITAMIN E

Vitamin E represents a group of tocol and tocotrienol derivatives that exhibit α-tocopherol activity and are found in high concentrations in vegetable oils (e.g., safflower, wheat germ, and sunflower oils).[7] The most commonly used form of vitamin E in supplementation trials is a synthetic blend of α-tocopherol stereoisomers referred to as dl-α-tocopherol or all-RAC-α-tocopherol. Although rare in human populations, studies in animal

models suggest that vitamin E deficiency is associated with impairments in cellular and humoral immunity.[5,8]

Supplementation of animals with vitamin E enhanced macrophage function[9,10] and CD4 T-cell activity.[11] Antibody production, including mucosal-associated secretory immunoglobulin A (sIgA), has also been observed to increase in response to supplementation in poultry.[10,12,13] Many of the animal studies, early supplementation studies in humans, and potential mechanisms for action have been reviewed previously by Meydani et al.[5,8]

The mechanisms for the immunomodulatory effects of vitamin E are hypothesized to be largely linked to its antioxidant activity. Vitamin E is a potent peroxyl scavenger and serves as the primary lipid-soluble, chain-breaking antioxidant in biological systems, protecting lipids from autoxidation. Therefore, it may confer protective benefits to the membranes of immune cells involved in the production of reactive oxygen species (ROS) via the respiratory burst. It has also been hypothesized that, in addition to its scavenging activities, vitamin E may reduce ROS production by interfering with protein kinase C phosphorylation, inhibiting NADPH oxidase assembly, and posttranscriptional modification of lipoxygenase enzymes.[14-16] In addition, vitamin E may attenuate age-related increases in prostaglandin E2 (PGE2), a T-cell suppression factor, via inhibition of peroxynitrite production and cyclooxygenase 2 (COX2) activity.[5,17] Incorporation of vitamin E into cellular membranes may stabilize immune cells by maintaining membrane fluidity and permeability. The antioxidant activity of vitamin E could protect omega-3 fatty acids, also immunomodulators, from auto-oxidation.[17-21]

Vitamin E Supplementation and Innate Immunity

The innate arm of the immune system is a rapidly activated first line of defense against pathogens. It is composed of phagocytic and natural killer (NK) cells that ingest and generate ROS to kill pathogens. Neutrophils, a specific group of phagocytic, polymorphonuclear leukocytes, are the most numerous cell type of the innate arm of the immune response and have been the focus of many vitamin supplementation studies, especially those involving antioxidants. Innate immunity also consists of a system of soluble mediators that include the complement cascade, acute-phase proteins, and cytokines that serve to neutralize pathogens and activate the more specific, adaptive arm of the immune function.

Circulating cell counts, proportions of cell types, and concentrations of other soluble mediators such as cytokines are often used as measures of innate immunity in nutritional supplementation studies. They are not con-sidered functional markers per se, as they do not necessarily measure responses of the immune system to specific stimuli (except perhaps in studies where participants are exposed to physical stresses).[22] The use of circulating markers is recommended, however, to establish the status of circulating immune cells of participants or as an indirect measure of cellular proliferation, differentiation, and activation. More specific measures of innate immunity used in nutritional studies are ex vivo markers of cell function in response to stimuli, such as degranulation, bactericidal capacity, and respiratory burst.

Distribution of Cell Types

In non-elderly populations, neither non-controlled trials[23,24] nor RCTs[25-27] support a role for vitamin E supplementation in altering circulating concentrations of total leukocytes, monocytes, neutrophils, phagocytic cells, or NK cells. In one placebo-RCT, adult men were randomized to receive either 536 mg/d vitamin E or placebo for 48 d, followed by a downhill running challenge[25] (Table 1). Among older participants (>50 years of age), the number of circulating neutrophils was significantly higher in the supplemented group compared with the placebo group the morning of the exercise challenge and 3 hours afterwards. No pre-supplementation values were reported, so baseline differences that may have existed prior to supplementation between the supplemented and placebo groups could not be accounted for. No effects of supplementation were observed in younger participants (2–29 years of age). Vitamin E supplementation (536 mg/d) for 2 months prior to a marathon had no effect on circulating monocytes, neutrophils, of NK cells in a RCT of 36 triathletes from the United States[26] (Table 1).

Cell Function

Some non-placebo-controlled studies of vitamin E supplementation in humans suggest that the functional activities of leukocytes and monocytes, as measured by enzyme activity and ROS production, could be influenced by supplementation. In adolescent and young adult southeast Asian males, supplementation with 300 mg/d vitamin E for 3 weeks significantly reduced bactericidal activity and acid phosphatase activity of leukocytes compared with baseline values.[28] Supplementation of both normolipidemic and hypertriglyceridemic patients with 402 mg/d vitamin E for 6 weeks significantly reduced superoxide production by polymorphonuclear leukocytes in response to phorbol-12 myristate 13-acetate (PMA), but significantly increased production in response to oxidized LDL.[29] These studies are limited, however, in their lack of a control group for comparison.

Table 1. Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]		P value[c]
				Placebo	*268 mg/d VE*	
United States[25]	VE (536 mg/d) vs. Placebo for 48 d	9 men; 22-29 y		*immediately pre and 6 hr, 1, 2, 5, 12 d post-exercise*	*immediately pre and 6 hr, 1, 2, 5, 12 d post-exercise*	
			Circulating neutrophils (x10^9/L)	3.0, 5.2*, 2.9, 3.3, 2.9, 3.6	2.8, 5.6*, 2.6, 2.3, 2.5, 2.1	≤0.05
			Superoxide concentration in neutrophil culture supernatants (μmol/ml)	0.4, 0.7, 0.8, 0.3, 0.5	1.1, 1.3, 0.7, 0.7, 1.1	NS
		12 men; 55-74 y		*Placebo*	*268 mg/d VE*	
				immediately pre, and 3 hr, 1, 2, 5, 12 d post-exercise	*immediately pre and 3 hr, 1, 2, 5, 12 d post-exercise*	
			Circulating neutrophils (x10^9/L) after exercise	2.9, 3.3, 2.8, 2.8, 2.8, 3.0	3.7†, 5.3*†, 3.9, 4.5, 3.4, 3.8	0.02
			Superoxide concentration in neutrophil culture supernatants (μmol/ml)	0.5, 0.6, 0.7, 1.2*, 1.3*	0.8, 1.1, 1.2, 1.4, 1.3	≤0.05
United States[26]	VE (536 mg/d) vs. Placebo for 2 mo prior to marathon	26 male and 10 female triathletes; mean age 37 y	Cell counts (x10^9/L)	*Placebo*	*536 mg/d VE*	
				immediately pre, post, 1.5 h post-race	*immediately pre, post, 1.5 h post-race*	
			Monocytes	"increased significantly after the race; patterns of change did not differ between groups"		
			Neutrophils			
			Total leukocytes	6.6, 16.8*, 15.4*	6.0, 17.2*, 15.5*	≤0.05
			NK cells	"increased significantly after the race; patterns of change did not differ between groups"		
			Plasma cytokine concentrations (pg/mL)			

Table 1. (Cont'd) Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]		P value[c]
			IL-10	5.7, 49.4, 28.3	4.3, 63.0, 37.2	NS
			IL-8	1.7, 18.4, 12.8	3.3, 26.0, 18.3	0.08[d]
			IL-6	89% higher post-race in the VE group than placebo		0.06[d]
			IL-1RA	107 % higher post-race in VE group than placebo		0.06[d]
			Salivary IgA secretion (µg/min)	195, 116, 131	186, 106, 109	NS
			Lymphocyte Counts (x10⁹/L)			
			Total Lymphocytes	2.1, 1.6, 1.4*	1.9, 1.7, 1.3*	≤0.05
			CD3 T cells	decreased significantly from baseline; patterns of change did not differ between treatment groups"		
			Activated T cells (CD69)			
			CD19 (B cells)	increased significantly from baseline; patterns of change did not differ between treatment groups		
South Africa[27]	VE (603 mg/d) vs. βC (40 mg/d) vs. Placebo for 6 wk	19 male and 41 female asymptomat ic smokers; mean age 33y		*Placebo* *BL, 4, 6, 12 wk*	*603 mg/d VE* *BL, 4, 6, 12 wk*	
			Leukocyte counts (x10⁹/L)	7.8, 7.1, 7.3, 6.7	8.1, 7.7, 8.2, 7.6	NS
			% change from baseline in mitogen-stimulated generation of ROS by blood phagocytes	*Placebo* *4, 6, 12 wk*	*603 mg/d VE* *4, 6, 12 wk*	
			PMA activated ROS generation	+14%, +8%, +6%	−29%*,†, −12%, +2%	0.005
			N-fMLP/CB-activated early response	−6%, −13%, −20%	−37%*, −25%*, −5%	≤0.05
			N-fMLP/CB-activated late response	+18%, +12%, +3%	−11%, +18%, +11%	NS

Table 1. (Cont'd) Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]				P value[c]
The Netherlands[30]	VE (268 mg/d) vs. Placebo for 2 y	128 male smokers; mean age 60 y	PMA-induced superoxide production by neutrophils after 2 y of supplementation	*Placebo*		*268 mg/d VE*		
			Cytochrome c reduction (nmol/min)	0.159		0.161		NS
			Chemiluminescence (x 10^-3 RLU/s)	201.5		197.0		NS
United States[31]	VE (60, 200, or 800 mg/d) vs. Placebo for 235 d	34 men and 44 women; ≥ 65 y		*Placebo*	*60 mg/d VE*	*200 mg/d VE*	*800 mg/d VE*	
			% C albicans killed by neutrophils at day 128	48%	38%	50%	54%	NS
			Lymphocyte subsets (% of total lymphocytes) Total T cells CD4 T cells CD8 T cells CD19 B cells	"No effect of VE supplementation"				
			Total nonspecific immunoglobulin concentrations (IgA, IgM, IgG)					
			Cutaneous DTH response[e]	*Placebo BL, 128d*	*60 mg/d VE BL, 128d*	*200 mg/d VE BL, 128d*	*800 mg/d VE BL, 128d*	
			diameter of induration	22, 24	24, 29*	17, 27*	17, 28*	≤0.05
			total number of positive responses[f]	3.5, 4.2	3.6, 5.1	3.6, 4.3	3.2, 5.4	NS

Table 1. (Cont'd) Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]				P value[c]
			median percent change in DTH response	+17%	+41%	+61%†	+49%	0.04
			Change in circulating concentrations of nonspecific immunoglobulins (IgG, IgM, IgA) from baseline to day 246	"not affected by VE supplementation				NS
			Vaccine Response					
			Anti-hepatitis B antibody titer	Placebo	60 mg/d VE	200 mg/d VE	800 mg/d VE	
			day 156 of study; prior to vaccine	4.0	4.0	4.0	4.0	NS
			day 186 of study (1 month after vaccine)	4.0	4.0	7.2	4.0	NS
			day 216 of study (1 month after booster 1)	4.6	6.0	12.1	4.4	NS
			day 246 of study (1 month after booster 2)	7.3	10.4	23.9*	9.2*	≤0.05
			Percent with detectable hepatits B titer one mo after last booster (day 246)	19%	28%	41%	42%	NS
			Anti-diphtheria antibody response (x-fold increase from before vaccination to day 246)	1.7	1.7	1.7	1.5	NS
			Anti-tetanus toxoid antibody (x-fold increase from before vaccination to day 246)	1.5	1.6	1.7*	1.2	0.04
			Antibodies against pneumococci	Placebo BL, 246 d	60 BL, 246 d	200 BL, 246 d	800 BL, 246 d	
			PN6B	0.8, 1.7*	0.8, 2.7*	0.5, 1.7*	0.8, 1.4*	≤0.05

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Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]				P value[c]
			PN14	1.0, 3.6*	1.2, 5.0*	1.3, 6.8*	0.7, 3.7*	≤0.05
			PN19F	1.3, 2.6*	0.9, 2.6*	1.1, 3.4*	1.6, 2.0*	≤0.05
United States[36]	VE (536 mg/d) vs. Placebo for 48 d.	21 men; 22-74 y	Plasma cytokine concentrations pre and 1, 2, 5, and 12 d post exercise (pg/mL)	*Placebo*		*536 mg/d VE*		
			IL-1β	"All of the plasma samples that [had] IL-1β immunoreactivity were in placebo group"				
			TNF-α	No difference between treatment arms at any time				
			Plasma IL-1β concentration 6 h post-exercise (ng/mL)	"All samples above detection limit were in placebo vs. none in VE"				
			LPS-induced cytokine production by PBMCs at baseline, 1, 2, 5, and 12 d post exercise (ng/mL)					
			IL-1β	At 24h post exercise, "secretion by cells from placebo increased 154% over baseline...Values in placebo remained higher than in VE to d12."				<0.05
			TNF-α	No difference between treatment arms at any time				
			IL-6	"concentrations of IL-6 in VE group were approximately half of the placebos immediately prior to exercise challenge"				0.06
				1-12 d post-exercise: "significantly less IL-6 in VE than placebo"				0.02
			PGE2	260% higher in VE compared to placebo at 1 – 12 d post-exercise				0.15

Table 1. (Cont'd) Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]		P value[c]
Brazil[37]	VE (800 mg/d) vs. Placebo for 180 d; All received anti-retroviral therapy	20 HIV+ men, 9 HIV+ women; 21-38y	Lymphocyte subsets	*Placebo BL, 60 120, 180 d*	*800 mg/d VE BL, 60 120, 180 d*	
			CD4 T cells (/mL)	310, 410, 430, 465	258, 393, 362, 379	NS
			CD8 T cells (/mL)	908, 826, 826, 826	898, 719, 782, 803	NS
			CD4 / CD8 ratio	0.37, 0.54, 0.54, 0.56	0.29, 0.53, 0.51, 0.52	NS
			HIV viral load (copies/mL)	148340, 3536, 865, 1191	113428, 4322, 1975, 1299	NS
			Lymphocyte viability			
			percent live lymphocytes	A 2.49 fold increase over time in VE vs. a 1.95 fold increase in the placebo group		0.05
			percent apoptotic lymphyocytes	significantly greater reduction in supplemented vs. placebo group		0.03
United States[40]	VE (800 mg/d) vs. Placebo for 30 d	9 men and 23 women ≥ 60 y	Mitogen induced cytokine production by PBMCs at baseline and day 30	*Placebo BL, 30d*	*400 mg/d VE BL, 30d*	
			IL-2 (Con A-induced, kU/L)	31.8, 37.5	35.6, 49.6*	<0.05
			% change in IL-2 production from baseline	-7%	67%*, †	<0.05
			IL-1 (endotoxin-induced)	"No significant change"		
			PGE₂ (PHA-induced; pmol/L)	8.3, 8.4	9.1, 8.5*	<0.05
			% change in PGE₂ production from baseline	"Greater in VE group than placebo"		<0.005
			Lymphocyte proliferation[g] (ccpm) in response to:			

Table 1. (Cont'd) Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]		P value[c]
			ConA	24478, 21954	20551, 23770*	<0.05
			SAC	"No significant change"		NS
			PHA	"No significant change"		NS
			Cutaneous DTH response[e]			
			Total positive reactions[f]	3.2, 3.3	2.5, 3.1*	<0.05
			Sum of indurations (mm)	16.5, 16.9	14.2, 18.9*	<0.05
			Sum of indurations (% change from baseline)	3.7	52.8†	0.04
			Serum immunoglobulin concentrations (IgG, IgA, IgM)	"No significant change was observed"		NS
The Netherlands[41]	VE (100 mg/d) vs. Placebo for 3 mo	48 men and 26 women; 67-85 y	Mean change from baseline in lymphocyte proliferation (n=52)	Placebo	100 mg/d VE	
			PHA-induced	0.15	0.03	NS
			Con-A-induced	0.13	0.06	NS
			Median change from baseline in concentration of antibodies against common antigens from baseline to 3 mo (n=74)			
			IgA Penicillium	0.01	-0.01	NS
			IgG Penicillium	0.1	-0.04	NS
			IgG4, egg protein	-0.1	0.02	NS
			IgG4, milk protein	-0.01	0.0	NS
			IgG4, wheat protein	0.0	0.0	NS

Table 1. (Cont'd) Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]			P value[c]
				Placebo BL, change from BL	50 mg/d VE BL, change from BL	100 mg/d VE BL, change from BL	
The Netherlands[43]	VE (50 mg/d or 100 mg/d) vs. Placebo for 6 mo	84 men and 84 women; 65-80 y	PHA-stimulated cytokine production by PMBCs after 24 wk				
			IL-2 (ng/L) 1 mg PHA/L	495, 421*	467, 355*	662, 420*	<0.01
			IL-2 (ng/L) 3 mg PHA/L	2780, 122	2496, 187	2989, 262	NS
			IFN-γ (10^3 U/L) 1 mg PHA/L	37, 1	37, -10*,†	37, -8	0.07
			IFN-γ (10^3 U/L) 3 mg PHA/L	44, -4	75, -17*	67, -15*	NS
			IL-4 (ng/L) 1 mg PHA/L	13, 2	12, 2.5*	10, 3*	<0.01
			IL-4 (ng/L) 3 mg PHA/L	35, -1	32, -1	36, 1	NS
				Placebo at 12, 24 wk	50 mg/d at 12, 24 wk	100 mg/d at 12, 24 wk	
			Change in DTH response[e] (all subjects) from baseline				
			Change in total positive reactions[f]	-0.2, 0.3	0.0, 0.4*	0.2, 0.8*,†	<0.05* 0.06†
			Change in cumulative DTH scores	1.0, 4.2*	2.7, 4.6*	2.2, 6.0*	<0.001
			Change in DTH response[e] from baseline among subjects with ≤ 2 reactions at baseline				
			Change in total positive reactions[f]	0.3, 0.5	0.3, 0.9	0.6, 1.3†	0.08
			Change in cumulative DTH scores	2.4, 3.7	2.3, 5.2	4.4, 8.0†	0.07
			Change in DTH response[e] from baseline to 24 wk among less physically active participants				

Table 1. (Cont'd) Randomized, Controlled Trials of Vitamin E (VE) in Relation to Immunological Parameters

Study site (ref)	Intervention groups	Population	End point[a]	Result for the indicated measure of effect[b]				P value[c]
				Placebo	30,000 IU VA	150 mg VE	VA + VE	
			Total positive reactions[f]	-0.2	0.3	0.3	0.9	NS
			Sum of indurations (mm)	1.6	4.7	4.7	7.2	NS
Turkey[48]	VE alone (150 mg/d) vs. VA alone (30,000 IU/d) vs. VA+VE vs. placebo with DPT vaccination at 2, 3, and 4 mo of age	89 infants	Titers of anti-tetanus IgG mIU/mL 2 mo 5 mo 16–18 mo	 268.4 880.6 314.4	 249.4 1126.6 314.7	 297.3 866.5 331.9	 157.2 1183 405.5	NS
			Percent of infants with protective anti-tetanus titers 2 mo 5 mo 16–18 mo	 83% 100% 92%	 87% 100% 92%	 86% 100% 88%	 76% 100% 92%	NS
				0 mg/d VE	200 mg/d VE	400 mg/d VE		
United States[49]	VE (0 mg/d, 200 mg/d, 400 mg/d) for 6 mo	103 patients in chronic care facility; age 24–104y	Antibody response to PVIV 1mo and 2d prior to and 1, 2, 3 mo post vaccine	No significant effect of supplementation on any endpoints measured, overall or stratified by age				

[a] Abbreviations used in table: βC: Beta-carotene, BL: Baseline measures taken prior to initiation of supplementation, ConA: Conconavalin A, DPT: Diphtheria / Pertussis / Tetanus toxoid, DTH: Delayed type hypersensitivity response, FBS: Fetal bovin serum, HIV: Human immunodeficiency virus, Ig: Immunoglobulin, IFN: Interferon, IL: Interleukin, IU: International units, LECL: Luminol enhanced chemiluminescence, LPS: Lipopolysaccharide, N-fMLP/CB: N-formyl-L-methionyl-L-leucyl-L-phenylalanine / cytochalasin, PBMC: Peripheral blood mononuclear cells, PGE2: Prostaglandin E2, PHA: Phytohemagglutinin, PMA: Phorbol myristate acetate, PMNLs: Polymorphonuclear leukocytes, PVIV: Polyvalent influenza vaccine, PWM: Pokeweed mitogen, RLU: Relative light units, ROS: Reactive oxygen species, SAC: Staphylococcus aureus Cowan I, TNF: Tumor necrosis factor, VA: Vitamin A, VE: Vitamin E.

[b] Values are means as presented in manuscripts; when actual values were unavailable, text corresponding to interpretations of results as provided in manuscripts was used.

[c] * Indicates that the P value is for change from baseline / pre-exercise levels; [†] indicates that the p value is for comparison of superscripted value with corresponding value in placebo group; NS indicates that all comparisons were p≥0.10; where applicable, P values calculated with Bonferroni adjustment for multiple comparisons as presented in the manuscripts are presented

[d] P value is for the treatment group*time interaction

[e] Cutaneous delayed type hypersensitivity response to 7 antigens including Tetanus and Diphtheria toxoids, Streptococcus (Group C), old tuberculin, Candida, Proteus, and Trichophyton

[f] Indurations ≥ 2 mm

[g] Measured as incorporation of ³H-thymidine after stimulation of peripheral blood lymphocytes with mitogens

Findings from randomized, placebo-RCTs suggest an influence of short-term, but not long-term, vitamin E supplementation on mitogen-stimulated ROS production. Among South African adult smokers, PMA and N-formyl-L-methionyl-L-leucyl-L-phenylalanine/cytochalasin (N-fMLP/CB)-induced generation of ROS by neutrophils was significantly reduced after 4 weeks of supplementation compared with baseline values in those randomized to receive vitamin E (603 mg/d).[27] Compared with placebo, only reductions in PMA-induced ROS production were statistically significant. Generation of ROS in response to PMA returned to baseline levels after 2 additional weeks of supplementation. Six weeks after supplementation ended, those in the supplemented group had significantly greater N-fMLP/CB-stimulated ROS production than the placebo group.

Longer-term, daily supplementation of smokers from the Netherlands (N = 128; 268 mg/d vitamin E for 2 years) had no effect on superoxide production by polymorphonuclear leukocytes as measured by cytochrome c reduction and chemiluminescence[30] (Table 1). In the RCT of nonsmoking men exposed to an exercise challenge,[25] superoxide production by neutrophils was unchanged in the supplemented group after the downhill running exercise, while significant increases in superoxide production were observed in the placebo group immediately following the exercise challenge. These findings may indicate a potential attenuation of exercise-induced ROS production by vitamin E, although the statistical significance of the treatment effect over time was not reported. In a separate, long-term supplementation study, 78 elderly subjects were randomized to receive placebo, 60, 200, or 800 mg/d vitamin E for 235 d. By day 128 of the study, there were no differences between treatment arms in the capacity of neutrophils to kill *Candida albicans*; it is not known whether there were differences in neutrophil function between treatments at baseline.[31] From a mechanistic viewpoint, it is unclear why reduced respiratory burst would be observed transiently with short-term but not long-term supplementation. No long-term studies have reported measures of respiratory burst or other functional indicators at intermediate time points.

Soluble Mediators

Tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and IL-6 are important inflammatory cytokines that mediate the recruitment of polymorphonuclear leukocytes and monocytes and influence leukocyte chemotaxis. Additionally, IL-8 and TNF-α promote degranulation and oxidative burst in neutrophils. Reductions in these cytokines could therefore influence cellular innate immune responses and oxidative damage to immune cells. In vitro studies suggest that vitamin E inhibits IL-1,[14] TNF-α,[32] and IL-6,[33] possibly via inhibition of protein kinase C and posttranscriptional modification of 5-lipoxygenase. In a non-controlled supplementation study in hypertriglyceridemic and normolipidemic men (N = 20), lipopolysaccharide (LPS)-induced production of TNF-α, IL-1β, and IL-8 by peripheral blood mononuclear leukocytes (PBML) was diminished after 6 weeks of supplementation (402 mg/d) compared with baseline.[29] Similarly, supplementation of healthy subjects and diabetic patients with 804 mg/d vitamin E for 3 months significantly reduced LPS-induced production of IL-6,[34] TNF-α,[32] and IL-1β[35] by monocytes compared with baseline values. Mitogen-stimulated cytokine production returned to baseline values after a washout period of 2 weeks to 2 months. Placebo controls were not available for comparisons in any of these studies.

Exhaustive exercise is associated with increased production of pro-inflammatory cytokines. The ability of vitamin E supplementation to influence exercise-induced increases in cytokines has been investigated in RCTs. Supplementation with 536 mg/d for 48 d attenuated post-exercise increases in LPS-stimulated production of IL-1β and IL-6 by PBMLs compared with placebo in a mixed population of adult and elderly participants[36] (Table 1). IL-6 concentrations were significantly lower in the vitamin E group compared with placebo immediately prior to the exercise and remained lower throughout the 12-day post exercise follow-up. Cytokine concentrations were not assessed prior to supplementation, so it is unclear whether baseline differences contributed to these findings. Post-exercise increases in plasma IL-6 levels were significantly greater in vitamin E-supplemented triathletes (536 mg/d) compared with those randomized to placebo; increases in plasma levels of IL-10 and IL-8 did not differ between the two treatment arms.[26] Thus far, the effects of vitamin E supplementation on inflammatory cytokine production in the absence of an exercise challenge have not been examined in RCTs. In the placebo-RCT study in triathletes,[26] the influence of vitamin E supplementation on exercise-induced changes in salivary IgA was investigated. Salivary IgA concentrations were significantly decreased immediately after the race compared with pre-race levels (Table 1). No differences were observed between the supplemented and placebo groups.

Vitamin E Supplementation and Adaptive Immunity

The adaptive arm of the immune systems utilizes T- and B-cell lymphocytes to neutralize pathogens or destroy infected cells in an antigen-specific manner. Measuring the response of the adaptive immune system to

vitamin supplementation is often accomplished by a combination of basal markers and ex vivo and in vivo measures.[22] Basal markers measure the ability of supplementation to alter the proportions or distributions of circulating lymphocytes or soluble mediators of the adaptive immune system. Ex vivo measures used in supplementation studies typically assess the ability of lymphocytes to respond to mitogens, and include such measures as lymphocyte proliferation, expression of activation markers, and cytokine production. In vivo integrated responses are considered to be the most suitable measures for assessing the effects of supplementation on adaptive immunity, and include the DTH response and the response to vaccines.[22]

Distribution of Lymphocytes

In a group of healthy men (n = 13) and women (n = 13) between 25 and 35 years of age living in Hong Kong,[23] daily supplementation with 233 mg/d vitamin E for 28 d significantly increased the proportion of total T-cells, the proportion of CD4 cells, and the CD4/CD8 ratio compared to baseline values. Also, supplementation of patients with advanced colorectal cancer (N = 12; 750 mg/d vitamin E for 14 d) significantly increased the number of CD8 memory T-cells and the CD4/CD8 ratio compared with baseline.[24] It is not possible to attribute a causal effect to vitamin E in these studies, given the absence of a control group.

Three RCTs have examined the effect of vitamin E supplementation on the proportions of circulating lymphocytes (Table 1).[26,31,37] Vitamin E supplementation of US triathletes for 2 months prior to a marathon did not significantly alter exercise-induced changes in lymphocyte subsets compared with placebo.[26] In a separate study, vitamin E supplementation (800 mg/d for 180 d) of HIV-positive men and women who were on antiretroviral therapy was not associated with changes in circulating CD4 and CD8 cells compared with the placebo group.[37] However, over the course of the follow-up period, supplementation significantly improved lymphocyte viability, as indicated by significant increases in the percent of live lymphocytes and decreases in the percent of apoptotic lymphocytes compared with placebo. Longer-term supplementation (235 days) of elderly men and women with 60 mg/d, 200 mg/d, or 800 mg/d was not associated with changes in the proportion of total T-cells, CD4, or CD8 cells.[31]

Lymphocyte Proliferation and Activation

Tests of lymphocyte proliferation are ex vivo measures of the ability of lymphocytes to become activated and replicate, typically after stimulation with a mitogen.

Lymphocyte proliferation can be used as a measure of cellular reactivity to a stimulus. In HIV-infected patients[38] and elderly populations,[39] reduced lymphocyte proliferation has been correlated with increased mortality, indicating that this measure is clinically relevant.[22]

Some studies suggest that vitamin E supplementation influences lymphocyte proliferation in a mitogen-specific manner. In a non-controlled study, healthy adults (N = 26) had significantly increased unstimulated lymphocyte proliferation and proliferation in response to both phytohemagglutinin (PHA) and LPS after 28 days of supplementation with 233 mg/d vitamin E compared with baseline values.[23] Additionally, supplementation significantly reduced lymphocyte hydrogen peroxide production compared with baseline.

Supplementation of older men and women in the context of a placebo-RCT with 800 mg/d vitamin E for 30 days significantly increased lymphocyte proliferation compared with baseline, but only in response to concanavalin A (ConA) stimulation; in the placebo group, ConA-induced lymphocyte proliferation decreased[40] (Table 1). No differences in lymphocyte proliferation in response to either *Staphylococcus aureus* Cowan I or PHA were observed.[40] ConA preferentially stimulates T-suppressor cells, while PHA is targets T-helper cells[17]; it is possible that the former are more sensitive to vitamin E. In another RCT of elderly persons (N = 83) from the Netherlands, supplementation with 100 mg/d vitamin E for 3 months had no effect on ConA or PHA-induced lymphocyte proliferation.[41] The dosage used in this study may have been insufficient to promote a response; the doses used in the trials that found significant effects were in excess of 200 mg/d. It is also feasible that discrepancies in the findings across studies were related to differences in the vitamin E status of the study populations at baseline or in the varying durations of the intervention. While findings of enhanced lymphocyte proliferation in response to vitamin E supplementation may seem inconsistent with the apparent lack of response with regard to lymphocyte counts, it should be noted that relatively few studies looked at both outcomes.[23,31] Furthermore, since these two measures represent different aspects of immunity, it is not necessarily expected that results from one would be indicative of the other.[22]

In a non-controlled intervention study of Chinese adults, vitamin E supplementation for 28 days did not alter the number of activated T-cells (CD25) expressing IL-2 receptors, a measure of lymphocyte activation.[23]

Soluble Mediators of Adaptive Immunity

Cytokine production by lymphocytes, specifically CD4-Th0 cells, is critical to regulating the intensity and direction of the adaptive immune response. For this

reason, assessment of cytokine production is often used as a measure of the functional capacity of lymphocytes.[22] IL-2 is a principal growth factor for T-cells. It is both produced by and acts on T-cells in a positive feedback manner. IL-2 and IFN-γ together promote a Th1 response and suppress a Th2 response, while IL-4 and IL-10 promote a Th2 response and suppress the Th1 response.[42]

In a small, non-controlled study, colorectal cancer patients (N = 12) supplemented with 750 mg/d vitamin E for 2 weeks had significantly increased PMA-induced IL-2 production by CD3, CD4, and CD8 T-cells and increased counts of IL-2-producing CD3, CD4, and CD8 cells compared with baseline measures.[24] Significantly increased production of IFN-γ by CD8 cells was also observed, whereas IL-10 production was unaffected. It should be noted that patients were also receiving selenium (60 μg/d) and vitamin C (50 mg/d) as part of the supplementation regimen to enhance the recycling of vitamin E and that the results could not be compared against a control group.

In an RCT of elderly men and women from the United States,[40] supplementation with vitamin E (800 mg/d for 30 days) was associated with significantly greater percent increase in ConA-stimulated IL-2 production by PBMLs compared with placebo (Table 1). In a RCT of elderly persons in the Netherlands, IL-2 production in response to low-dose PHA increased significantly from baseline values in those randomized to receive placebo, 50 mg/d vitamin E, and 100 mg/d vitamin E for 6 months.[43] Although there was a trend for greater increases in the vitamin E-supplemented groups compared with placebo, the differences were not statistically significant. Production of IFN-γ was significantly decreased from baseline among those supplemented with 50 mg/d compared with the placebo group. No effects of supplementation on IL-4 production were observed[43] (Table 1). The effect of vitamin E supplementation on mitogen-stimulated cytokine production by lymphocytes in younger populations has not been reported.

PGE2 is a metabolite of arachidonic acid that inhibits T-cell proliferation. Age-associated increases in the production of PGE2 are hypothesized to contribute to immunosuppression in elderly populations. Animal studies indicate that vitamin E can attenuate PGE2 production by macrophages of old mice.[44,45] In the RCT among elderly men and women in the United States,[40] vitamin E supplementation (800 mg/d) was associated with significantly reduced PGE2 concentrations after 30 d compared with placebo. In contrast, in a separate RCT of nonsmoking younger men (22–74 years of age; N = 21) 536 mg/d vitamin E for 48 d resulted in a non-statistically significant 260% increase in PGE2 concentrations compared with placebo.[36] This difference was not af-

fected by the age of the participants or by an exercise challenge. The discrepancies between these two studies may be attributed in part to the effect of different supplementation doses on plasma vitamin E levels. In the study of older subjects,[40] plasma concentrations increased 177% compared with 64% among participants in the study of younger men.[36]

Delayed-Type Hypersensitivity Response

Cell-mediated immunity neutralizes intracellular pathogens via direct killing of infected cells by cytotoxic CD8 T-cells and by CD4-Th1 activated macrophages. DTH is a commonly used in vivo assessment of cellular immunity. It is an antigen-specific, T-cell-mediated response that involves the recruitment and activation of effector macrophages.[42] A reduced DTH response has been associated with increased morbidity and mortality in elderly populations.[46,47]

In a non-controlled intervention study, supplementation of healthy adults and children with 300 mg/d vitamin E for 3 weeks did not alter the DTH response to PHA compared with baseline.[28] RCTs investigating the effects of vitamin E supplementation on the DTH response have been conducted in older populations. In these studies, both short-term (30 d) and long-term (235 d) supplementation with a range of doses (100–800 mg/d vitamin E) significantly increased the DTH response to 7 antigens compared with placebo groups (Table 1).[31,40,43] In one of these studies,[43] increases were more substantial in anergic and less physically active elderly subjects.

Humoral Immunity

The humoral arm of the adaptive response involves production of antibodies by B-cells in response to specific pathogenic stimuli; this response can be facilitated via CD4 T-cell-dependent or -independent mechanisms. Basal markers of humoral immunity include circulating concentrations of immunoglobulins; however, it has been noted that concentrations of immunoglobulins are not responsive to dietary changes and therefore may not be a reliable measure of the effects of vitamin supplementation on adaptive immunity in healthy populations.[22] A more reliable measure of the effect of supplementation on humoral immunity is the in vivo response of the immune system to vaccination.

Circulating Immunoglobulins

In two RCTs conducted among elderly US men and women, neither short-term (30 days)[40] nor long-term (235 days)[31] supplementation with 60, 200, or 800 mg/d

vitamin E had significant effects on the circulating concentrations of nonspecific immunoglobulins (IgG, IgM, IgA) (Table 1). Similarly, supplementation of elderly subjects from the Netherlands with 100 mg/d vitamin E for 3 months did not alter circulating levels of IgG or IgA specific to four strains of penicillin or IgG4 specific to egg, wheat, or milk proteins[41] (Table 1).

T-Cell-Dependent Vaccine Response

In a supplementation trial of elderly US men and women (N = 78) with 60, 200, or 800 mg/d vitamin E or placebo for 235 days,[31] standard doses of hepatitis B, diphtheria, and tetanus toxoid vaccines were administered on day 156 of supplementation, and additional hepatitis B boosters were administered on days 186 and 216 (Table 1). Supplementation with both 200 and 800 mg/d vitamin E produced significantly higher antibody titers 1 month after the second and third hepatitis B boosters compared with baseline, whereas no significant increases were observed in the placebo group. The treatment effect, however, was not statistically significant at any time point. Subjects supplemented with 200 mg/d, but not with placebo or 800 mg/d, had significantly higher antibody titers to tetanus toxoid at day 246 of the study compared with baseline. Vitamin E did not have significant effects on the response to diphtheria vaccine.[31]

A 2×2 factorial randomized study examined the effects of supplementation with vitamin A alone, vitamin E alone (150 mg), vitamin E + vitamin A, or placebo on the response to diphtheria-pertussis-tetanus toxoid vaccination in infants when administered at 2, 3, and 4 months of age.[48] Vitamin A and vitamin E supplements were given daily for 3 days and 1 day after each vaccination, respectively (Table 1). Anti-tetanus IgG titers and the percent of infants with protective anti-tetanus titers were determined at 2, 5, and 16 to 18 months of age. Compared with the placebo arm, neither supplementation with vitamin E alone or vitamin E provided in combination with vitamin A altered the infants' antibody response or acquisition of protective titers to tetanus toxoid.[48] In a separate RCT, patients in a chronic care facility were supplemented with 0, 200, or 400 mg/d vitamin E for 6 months.[49] One month after the initiation of supplementation, patients were administered a single intramuscular injection of polyvalent influenza vaccine. Supplementation had no effect on serum antibody titers measured at 1, 2, or 3 months post-vaccination (Table 1).

T-Cell-Independent Vaccine Response

The effect of vitamin E supplementation on antibody response to pneumococcal polysaccharide vaccine was assessed by Meydani et al.[31] in their study of 78 elderly persons in the United States. By the end of the study (246 days), serum antibody titers were significantly increased from baseline values in all groups. However, no significant differences were observed between the treatment groups and the placebo group (Table 1).[31]

Vitamin E Supplementation and Clinical Outcomes

The majority of RCTs that have examined the effects of vitamin E supplementation on specific clinical outcomes have focused on morbidity related to respiratory infections.

Respiratory Infections

The effect of vitamin E supplementation on respiratory infections have been examined primarily in elderly populations. In an RCT (N = 617), elderly nursing home residents (≥65 years of age) in the United States were supplemented with 134 mg/d vitamin E for one year and followed periodically to determine the incidence, severity, and duration of respiratory infections, including the common cold, influenza-like illnesses, pharyngitis, otitis media, sinusitis, acute bronchitis, and pneumonia.[50] Participants in the vitamin E group who completed the study were approximately 10% less likely to acquire one or more respiratory tract infections and 20% less likely to experience one or more general upper respiratory tract infections compared with those receiving placebo. Additionally, those assigned to vitamin E supplementation had a significantly lower incidence of common colds (RR = 0.80; 95% CI 0.64–0.98).[50] Vitamin E supplementation had no impact on the incidence of lower respiratory tract infections or the duration or type of all respiratory infections. In a separate randomized, placebo-controlled, 2x2 factorial study, 652 elderly persons from the Netherlands (>60 years of age) were randomized to receive one of four treatments daily over 15 months: placebo, vitamin E (200 mg/d), multiple micronutrients, or multiple micronutrients + vitamin E.[51]

Comparisons between the vitamin E only and placebo groups were nonsignificant for the incidence and severity of acute respiratory infections. However, when the vitamin E and multiple micronutrient + vitamin E groups were combined and compared with those not receiving vitamin E, the severity of infections, as indicated by the presence of co-infections, duration, fever, and activity restriction, was apparently increased in those who received vitamin E compared with those who did not. Population heterogeneity between these two studies may have contributed to differences in the findings. Although both groups were similar with respect to base-

line vitamin E and smoking status, participants in the US study[50] were institutionalized, while the participants in the Netherlands trial[51] were free-living. Additionally, the participants in the US trial were approximately 10 years older (~84 vs. ~73 y), more likely to have type 2 diabetes (~20% vs. ~10%) and cardiovascular disease (~33% vs. ~15%), and more likely to be female (~73% vs. ~50%). It should also be noted that in the Netherlands trial,[51] no distinction was made between upper and lower respiratory tract infections, so pneumonia and the common cold could have been counted as similar outcomes.

In a large study of male smokers (N = 21,796) drawn from the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study,[52] long-term supplementation with a 50 mg/d vitamin E for 6 years was not associated with the overall incidence of common colds.[53] Although not statistically significant, men supplemented with vitamin E who engaged in regular strenuous physical exercise had an apparent 10% greater risk of the common cold compared with those in the placebo group. Among older participants (≥ 65 years of age), the incidence of colds was slightly lower in the vitamin E group; the greatest reduction was observed in older urban residents who were light smokers (<15 cigarettes/d).[54] Supplementation was associated with a statistically significant 35% reduction in pneumonia risk in men who initiated smoking after 21 years of age.[55]

Other Infectious Diseases

No RCTs of vitamin E supplementation alone have been reported with respect to malaria, diarrhea, tuberculosis, or other infectious diseases.

Vitamin E Supplementation Summary

Current evidence from RCTs does not support a consistent role for vitamin E supplementation in influencing counts of innate immune cells, with the exception of increased neutrophil counts in older populations. While short-term supplementation (4 weeks) with vitamin E appeared to reduce ROS production, no effects have been observed with longer-term supplementation. It is unclear why supplementation would be associated with transient reductions in ROS production that returned to normal with additional supplementation. The apparent inconsistency in findings with respect to the effects of supplementation on ROS production may be related to methodological differences or population heterogeneity. Although non-controlled intervention studies suggested that vitamin E supplementation may reduce concentrations of pro-inflammatory cytokines, RCTs examining these effects in populations that are not undergoing exercise challenges are lacking.

Some studies suggest that vitamin E supplementation enhances cell-mediated adaptive immunity in older populations. In RCTs conducted in the elderly, vitamin E supplementation at 400 mg/d or more was associated with increased ConA-stimulated IL-2 production, increased lymphocyte proliferation in response to ConA, and enhanced DTH response. Taken collectively, these findings suggest that vitamin E supplementation may enhance a Th1-cell-mediated response. In vitro experiments, non-controlled supplementation studies, and one RCT among elderly persons support a role for vitamin E supplementation in reducing age-associated increases in PGE2 production. Because high concentrations of PGE2 inhibit T-cell function and proliferation, vitamin E-associated decreases in PGE2 production may offer a mechanistic explanation for enhanced immune function with vitamin E supplementation in the elderly. The effects of supplementation on various parameters of adaptive immunity in non-elderly populations are less clear due to a lack of RCTs.

The ability of vitamin E supplementation to enhance cell-mediated immunity in the elderly could lend mechanistic support to the observed benefits of vitamin E against upper respiratory infections that may be caused by viral agents. One additional mechanism could be related to the high oxygen content of lung tissues, which increases their susceptibility to damage from infection-associated inflammation and ROS production. Hypothetically, vitamin E-mediated reductions in the generation of ROS could protect lung tissue from damage induced by inflammation, and potentially reduce the duration or severity of respiratory infections. Findings from the three published RCTs, however, do not support a role for vitamin E supplementation in reducing the duration or severity of respiratory infections in elderly populations or smokers. Reductions in the incidence of the common cold were observed in two of the three studies conducted in elderly persons. Research in children, in whom respiratory illnesses are also frequent and potentially life-threatening, is lacking. With the exception of an enhanced response to tetanus, findings from a limited number of studies do not suggest a role for vitamin E supplementation in humoral immunity.

SUPPLEMENTATION WITH VITAMIN C

Ascorbic acid is a 6-carbon lactone that readily oxidizes in aqueous solution to its di-keto form, dehydroascorbic acid.[56] Both forms contribute to the biological activities of vitamin C. Vitamin C is a potent and versatile antioxidant, effectively quenching free radicals and protecting cell membranes and intracellular proteins from oxidative damage. In addition, vitamin C enhances the utilization of other nutrients. It facilitates iron ab-

sorption, the regeneration of vitamin E, and the use of calcium in bone.[57] Vitamin C-rich foods include cherries, black currants, cruciferous vegetables, citrus fruits, strawberries, and red bell peppers.

Studies in cell cultures and animal models suggest that vitamin C influences a wide range of immune-related functions of both the innate and adaptive arms. High circulating levels of serum vitamin C are associated with enhanced antibody response,[58,59] neutrophil function,[60,61] and mitogenic response[62] in several animal models. Ascorbate has also been shown to inhibit several viruses in vitro, including herpes virus,[63] rhinoviruses,[64,65] and HIV.[66,67] Below we review the impact of vitamin C supplementation on parameters of innate and adaptive immunity and discuss potential correlations between the observed effects and clinical outcomes.

Vitamin C Supplementation and Innate Immunity

The majority of studies on the impact of vitamin C supplementation on parameters of innate immunity have been conducted in adult endurance athletes and in populations with chronic illnesses. Many of these studies have focused on neutrophil counts and functions as the key end points. Exercise elicits increases in the number of neutrophils and their capacity to generate ROS. The excessive production of ROS following exercise may damage neutrophils and impair their function, potentially contributing to immunosuppression for a period following strenuous exercise. Because neutrophils serve as a first line of defense against viral pathogens, the influence of exercise on neutrophils may explain the increased risk of viral upper respiratory tract infections that has been noted to occur immediately following strenuous exercise challenges.[68,69] Due to its antioxidant capabilities, vitamin C is hypothesized to attenuate exercise-induced ROS production, oxidative stress, and inflammation, and thus to potentially ameliorate exercise-induced immunosuppression.[68,70-72] Supplementation with vitamin C may protect against exercise-induced damage by ROS and preserve the functional capacity of neutrophils. Likewise, the antioxidant capacity of vitamin C could alleviate the oxidative stress and its associated neutrophil damage that follow acute and chronic diseases and trauma.

Distribution of Cell Types

In a small placebo-controlled study, 16 endurance runners were supplemented with either 1000 mg/d vitamin C or placebo for the week preceding, the day of, and 2 days after an ultramarathon.[73] Supplementation was associated with significantly greater increases in circu-

lating monocytes compared with placebo immediately post-race, but these differences were not sustained 1 hour after the race. No differences were observed in the proportions of circulating leukocytes or neutrophils. This study was presumably not randomized, and differences in dietary intakes of other potentially immunoregulatory nutrients existed between the treatment arms. In a later trial by the same research team and with a similar, presumably non-randomized design, 45 endurance runners received placebo, 500 mg/d vitamin C, or 1500 mg/d vitamin C.[74] The supplements had no effect on circulating leukocytes, neutrophils, or monocytes at either dose.

RCTs have been conducted in endurance runners and apnea divers to investigate the effects of vitamin C supplementation on the distributions of circulating innate immune cells (Table 2; please go to www.ilsi.org/Publications/NutritionReviews/). Apnea diving is characterized by repeated episodes of hypoxia and reoxygenation, which induce oxidative stress. In a small, randomized, placebo-controlled, double-blind, crossover study, professional apnea divers (N = 7) were supplemented with 1000 mg/d vitamin C for 7 days prior to a 4-hour period of apnea diving exercises.[75] Supplementation was associated with significantly higher neutrophil counts after the diving exercises and after 1 hour of recovery (Table 2; please go to www.ilsi.org/Publications/NutritionReviews/). In another small, randomized, crossover study of endurance-trained males (N = 9), supplementation with 1000 mg/d for 2 weeks significantly attenuated exercise-induced increases in the concentrations of circulating leukocytes and neutrophils compared with placebo.[76] In two additional placebo-controlled, randomized studies in ultramarathoners (N = 28)[77] and marathoners (N = 12),[78] vitamin C supplementation had no effects on neutrophil, monocyte, NK cell, or total leukocyte counts after a 12-hour and a 2.5-hour running challenge, respectively. These studies were similar in supplement dosage (1000–1500 mg/d) and duration of the intervention prior to the exercise challenge (7–9 days). The results of these trials may have varied in part due to differences in sample sizes, the types and extent of exercise challenges, the duration of supplementation, and the intake of other nutrients during the exercise trials. Furthermore, despite the randomized, controlled nature of these studies, it is not possible to completely rule out chance as a possible explanation of their findings, given the relatively small sample sizes.

Cell Function

Early non-controlled studies of vitamin C supplementation in populations with underlying illnesses such as asthma, tuberculosis, and chronic granulomatous dis-

ease and those who experienced trauma were reviewed by Jariwalla and Harikeh.[79] These studies suggested that vitamin C supplementation enhanced neutrophil motility, chemotaxis, and post-phagocytic metabolic activity, including neutralization of phagocytic-derived oxidants.

More recently, a placebo-controlled study in patients with prostatic hyperplasia (N = 120) showed that, compared with the placebo arm, an intravenous infusion with 500 or 1000 mg/d vitamin C for 3 days in combination with standard therapy significantly increased the phagocytic activity of neutrophils 6 days after surgery.[80] Functional capacity of neutrophils as measured by the restoration of nitroblue of tetrazolium test, was significantly increased in the 500 mg/d arm, but not the 1000 mg/d arm, compared with the placebo group. These findings should be interpreted with caution, however, as the study was presumably not randomized. No effects of supplementation on neutrophil function were reported in a non-controlled study of 10 athletes supplemented with 2000 mg/d of vitamin C for 1 week prior to participating in a biathlon.[81]

Some RCTs involving athletes have examined antioxidant capacity and ROS production of neutrophils. In the small study with apnea divers, vitamin C supplementation attenuated hypoxia-induced increases in neutrophilic glutathione (GSH) peroxidase but not in glutathione reductase or superoxide dismutase (SOD)[75] (Table 2; please go to www.ilsi.org/Publications/NutritionReviews/). Glutathione peroxidase and catalase neutralize hydrogen peroxide. Vitamin C may have reduced the production of hydrogen peroxide and thus the necessity for upregulation of these enzymes by protecting lipids from oxidation. In the small (N = 9), crossover trial of endurance athletes[76] (Table 2; please go to www.ilsi.org/Publications/NutritionReviews/), the capacity of neutrophils to generate ROS in response to N-fMLP immediately following a 2.5-hour cycling exercise was apparently increased from pre-exercise levels in the supplemented athletes and decreased in the placebo group. At 1 hour post-exercise, the oxidative capacity of neutrophils was below pre-exercise levels for both groups. Exercise-induced reductions in neutrophil degranulation were not significantly attenuated by supplementation with 1000 mg/d vitamin C. The study had limited statistical power to detect significant effects of supplementation.

Soluble Mediators

Repeated exercise-associated increases in inflammatory cytokines may contribute to immunosuppression in endurance athletes. In the previously described studies with ultramarathon runners, daily supplementation with vitamin C (500 or 1500 mg) for 7 days did not influence exercise-induced changes in plasma cytokine concentra-

tions (IL-6, TNF-α, IL-10, IL-1 receptor antagonist [IL-1RA], IL-8).[73,74,82] In one of the studies, when comparisons were made after collapsing the 500 mg and placebo groups, plasma concentrations of IL-1RA, IL-1, and IL-10 were significantly decreased immediately post race compared with pre-race in those supplemented with 1500 mg/d.[82] Non-statistically significant trends for decreased IL-6 and IL-8 were observed in the 1500 mg/d group. Supplementation with vitamin C (500 or 1500 mg) significantly attenuated exercise-induced increases in circulating cortisol immediately post-race compared with placebo in two of the studies.[73,82] In one study,[73] the exercise-induced acute-phase response, measured as the serum concentration of C-reactive protein, was significantly higher in the supplemented group (500 mg/d) compared with placebo immediately post-race; this enhancement was maintained for 48 hours post-race, potentially indicating a heightened inflammatory response in the supplemented group.

In an RCT of endurance runners (N = 28), supplementation with 1500 mg/d vitamin C for 8 days prior to an ultramarathon did not attenuate exercise-induced increases in plasma concentrations of IL-6, IL-1RA, IL-10, or IL-8.[77] Among Chinese patients with acute pancreatitis (N = 84), those randomized to receive 10,000 mg/d intravenous vitamin C for 5 days had significantly greater decreases from pre-therapy values for circulating TNF-α, IL-6, and IL-8 compared with patients supplemented with 1000 mg/d.[83] Differences between the two study groups with respect to dosages, underlying health conditions, and stress exposures (trauma vs. exercise) may have contributed to the differences in findings between these two RCTs.

In a non-controlled study of five healthy adult men, no apparent effects of vitamin C supplementation (1000–3000 mg/d for 3 weeks) on circulating concentrations of complement proteins were observed.[84] In the RCT with ultramarathoners (N = 28) supplementation with 1500 mg/d vitamin C for 7 d prior to an ultramarathon did not attenuate exercise-induced changes in salivary IgA concentrations, sIgA secretion, or the sIgA/saliva protein ratio compared with placebo.[85]

Vitamin C Supplementation and Adaptive Immunity

A role for vitamin C supplementation in enhancing lymphocyte function is supported by in vitro and animal studies. The following section reviews vitamin C supplementation studies in humans and its effects on parameters of adaptive immunity, including lymphocyte counts and functions and measures of cell-mediated and humoral immunity.

Distribution of Lymphocytes

The effects of vitamin C supplementation on lymphocyte subsets have been studied in patients with acute pancreatitis and endurance athletes. In the Chinese study of patients with acute pancreatitis (N = 84), significant increases from baseline were observed in the proportion of CD4 cells and the CD4/CD8 ratio among those randomized to receive 5 days of 10,000 mg IV vitamin C compared with patients randomized to receive only 1000 mg/d (Table 2; please go to www.ilsi.org/Publications/NutritionReviews/).[83] In contrast, vitamin C supplementation of up to 1500 mg/d in marathoners and ultramarathoners did not alter the number of circulating T-cells, B cells, or total lymphocytes as reported in two RCTs (Table 2; please go to www.ilsi.org/Publications/NutritionReviews/).[77,78]

Lymphocyte Proliferation and Activation

Despite no apparent effect on cell counts, there is some evidence from non-controlled studies that suggests vitamin C supplementation may enhance mitogen-induced lymphocyte proliferation. In a study of 10 children with bronchial asthma, PHA and ConA-stimulated lymphocyte proliferation was significantly increased from baseline after 1, 3, and 6 months of 1000 mg/d vitamin C.[86] Similarly, supplementation of five healthy adults with up to 3000 mg/d vitamin C for 3 weeks significantly increased PHA and ConA-induced lymphocyte proliferation.[84] No control groups were available in either study for comparison purposes.

Results from RCTs also indicate a role for vitamin C supplementation in enhancing lymphocyte proliferation. In a randomized, placebo-RCT of nonathletic healthy elderly persons (N = 20), PHA and ConA-induced lymphocyte proliferation was significantly greater after 1 month of supplementation (500 mg/d injections) compared with baseline and placebo.[87] In the RCT of ultramarathoners from the United States (N = 28), lymphocyte proliferation in response to PHA was higher in those supplemented with 1500 mg/d vitamin C for 7 days immediately prior to and during the race compared with placebo, although this difference was not statistically significant.[77] However, when lymphocyte proliferation was adjusted for changes in T-cells, a borderline statistically significant treatment effect was observed ($P = 0.07$).

Mediators of Adaptive Immunity

In the study of 28 ultramarathoners, PHA-stimulated production of IL-2 and IFN-γ by lymphocytes was examined immediately prior to, during, and immediately following the ultramarathon.[77] Production of the two cytokines decreased significantly both during and after the race compared with pre-race measures. There were no significant differences in cytokine production between the supplemented and placebo groups at any time.

Delayed-Type Hypersensitivity Response

The DTH response has been shown to be diminished in humans with experimentally induced vitamin C deficiency.[88] To date, two studies have examined the influence of vitamin C supplementation on DTH response. One study was a non-controlled supplementation study in patients undergoing hemodialysis[89]; the second was an RCT in healthy elderly men and women[87] (Table 2; please go to www.ilsi.org/Publications/NutritionReviews/). Vitamin C supplementation had no effect on the DTH response in either of these studies.

Humoral Immunity

Small early studies in both healthy adult volunteers[84] and asthmatic children[84,86] did not offer evidence for a role of vitamin C supplementation in altering the concentrations of circulating IgG, IgM, or IgA. Likewise, a randomized, placebo-RCT in healthy elderly persons showed no effect of 500 mg/d intramuscular vitamin C on circulating levels of IgA, IgG, or IgM from baseline to day 30 compared with placebo.[87] It has been argued that circulating immunoglobulins may not be responsive to dietary interventions except in conditions that may impair protein synthesis, such as protein-energy malnutrition. Detection of antigen-specific antibodies in response to vaccines is considered a more reliable measure of the effects of nutritional interventions on adaptive immunity.[22] However, to date, no studies have evaluated the effects of vitamin C supplementation on vaccine response.

Vitamin C Supplementation and Clinical Outcomes

Respiratory Infections

Vitamin C has been extensively examined for its potential to modulate morbidity due to respiratory infections, especially the common cold, since Linus Pauling first suggested a beneficial effect in 1971.[90-92] Studies conducted thereafter, mostly in Western populations, have reported conflicting findings, and the benefits of routine vitamin C supplementation on reducing the incidence, severity, and duration of the common cold have been debated.[93-95] The findings from published placebo-RCTs that examined the influence of both prophylactic and therapeutic vitamin C supplementation in excess of

200 mg/d on the incidence, severity, and duration of the common cold were recently evaluated in a Cochrane review.[95]

Vitamin C supplementation (> 200 mg/d for 3 weeks to 6 months) had no effect on the incidence of common colds in the general population, as shown by a review of 32 community prophylaxis trials.[95] However, it was noted that vitamin C supplementation significantly reduced the incidence of common colds by approximately 40% among subjects exposed to physical stress or cold, for example, marathon runners or military troops in cold climates.

Thirty studies examined the effects of vitamin C supplementation on the duration of illness. Supplementation was associated with 8% and 14% reductions in illness duration in adults and children, respectively. The majority of these trials utilized a 1000 mg/d dose and thus estimation of dose-response was not possible. Vitamin C supplementation was associated with small reductions in the severity of episodes when defined as the number of days "confined to home" or "days off work or school," but not when severity was summarized as a symptom severity score.

Three studies that examined the effects of prophylactic vitamin C supplementation on reducing the incidence, severity, and duration of experimentally induced infection were not included in the pooled estimates for prophylactic studies.[64,96,97] One study[97] reported that fewer participants in the vitamin C arm became ill with common cold symptoms, whereas two of the studies noted a reduction in symptom severity[64,97] compared with placebo groups. The third study found no effect of vitamin C supplementation on common cold morbidity.[96]

Therapeutic administration of vitamin C at the onset of cold symptoms had no effect on either duration or severity of symptoms, with the exception of one study that used a single dose of 8000 mg administered on the first day of symptoms.[98] Findings from this review indicate that vitamin C is not effective at reducing the incidence of common colds in the general population. Its effectiveness at alleviating the duration and possibly the severity of symptoms is limited to prophylactic use, with the greatest benefits observed in children. The authors noted the lack of studies that have investigated the potential risks and benefits of prophylactic and therapeutic supplementation with doses in excess of 3000 mg/d and 6000 mg/d, respectively.

A recent RCT of prophylactic vitamin C supplementation was conducted in Japanese men and women with atrophic gastritis.[99] Three hundred and five eligible subjects were randomized to receive either low-dose (50 mg/d) or high-dose (500 mg/d) vitamin C for 5 years. While the risk of experiencing one cold was not affected,

the risk of contracting three or more colds during the 5-year follow-up period was reduced by approximately 70% in those receiving 500 mg/d vitamin C ($P = 0.04$). In those randomized to the 500 mg/d group, the mean number of days with a runny nose was significantly greater than in the 50 mg/d group (0.9 vs. 3.1 days, respectively). There were no treatment differences on total duration of the colds. One limitation of the study is that the outcome definition relied on each subject's interpretation of getting a common cold. In addition, changes in biochemical measurements of vitamin C status over time were small and suggested that compliance with the study regimen may not have been optimal.

Two RCTs have examined the effect of vitamin C supplementation on the incidence of pneumonia among populations living in crowded conditions. Vitamin C supplementation at 2000 mg/d for 2 months was associated with an 85% reduction in the incidence of pneumonia in US military recruits compared with placebo.[100] In the former Soviet Union, the therapeutic administration of vitamin C (300 mg/d) to military personnel who contracted influenza significantly reduced the incidence of pneumonia by 80% compared with the control group. Studies in other populations at high risk of pneumonia, such as institutionalized elderly, have not been conducted.

Early studies indicated that tolerance to large doses of vitamin C increased in patients with infections, and that supplementation to bowel tolerance reduced the severity and duration of infections.[101-104] While some researchers propose the use of vitamin C supplementation titrated to bowel tolerance to treat and/or prevent respiratory infections, placebo-RCTs using doses in excess of 6000 and 8000 mg/d for prophylaxis and therapeutic studies respectively, have not been published. The range of doses in the Cochrane review summarized above[95] was not sufficiently wide to estimate a dose-response effect. However, in two placebo-controlled, prophylaxis studies, doubling of dose (3000 to 6000 mg/d in adults[105] and 1000 to 2000 mg/d in children[106]) resulted in an approximate doubling of benefit with respect to reductions in the duration of common cold symptoms. In addition, although the pooled estimate from therapeutic trials was not statistically significant, a significant reduction in the duration of symptoms was observed in a study in which participants received 8000 mg vitamin C on the first day of supplementation.[98]

Other Infectious Diseases

Studies in neonatal calves suggest a role for supplementation in prevention of diarrhea.[107,108] However, the effects of supplementation of humans with doses that do not exceed bowel tolerance on diarrhea morbidity have

not been assessed. In vitro studies indicate that vitamin C might inhibit replication of HIV,[66,67,79] and early non-controlled studies in HIV-positive individuals suggested that mega-dose supplementation (50–200 g/d) was associated with reduced frequency and severity of opportunistic infections.[102] However, RCTs of vitamin C alone have not been conducted to test these hypotheses.

Vitamin C Supplementation Summary

Whether vitamin C supplementation has an effect on counts of innate immune cells is uncertain. RCTs have been limited by low statistical power. On the other hand, it has been suggested that the proportions of circulating immune cells may not be a sensitive measure to assess nutritional-related changes in immune function, because typically a very low proportion of immune cells are in circulation at any given time.[22]

Non-randomized studies suggest a role for vitamin C in improving neutrophil motility, chemotaxis, and bactericidal activity in populations with chronic illnesses, but evidence from RCTs is lacking. In athletes, small RCTs investigating the short-term effects of vitamin C supplementation on neutrophil antioxidant capacity and ROS production have yielded conflicting results. RCTs do support a role for vitamin C supplementation in attenuating trauma- but not exercise-associated increases in serum concentrations of pro-inflammatory cytokines. Studies investigating the effects of vitamin C supplementation on cytokine production, sIgA production, and complement activity are limited to generate conclusions.

Lymphocyte counts do not appear to be influenced by vitamin C supplementation. However, characterization of the effects of supplementation on lymphocyte subsets such as CD4 T-cells, CD8 T-cells, and B cells is limited. Despite an apparent lack of effect on lymphocyte counts, evidence from small RCTs in athletes and elderly populations suggests that vitamin C supplementation may enhance lymphocyte proliferation. Two studies in elderly populations failed to provide support for a role of vitamin C supplementation in enhancing the DTH response. Similarly, supplementation does not appear to influence concentrations of circulating immunoglobulins. Additional findings related to functional measures of adaptive immunity (e.g., vaccine response) have not been reported.

Several controlled studies suggest a small benefit of vitamin C supplementation at doses ranging from 1000 to 8000 mg/d in reducing the duration, but not the incidence, of respiratory infections; a greater benefit seems apparent in children. Among subjects regularly engaged in strenuous physical activity or who live in crowded situations, vitamin C supplementation appears to reduce the incidence of common colds and pneumo-nia. It has been suggested that strenuous exercise and physical stress skew the immune system toward a pre-dominantly Th2 immune response.[69] Individuals experiencing physical stress may therefore be at greater risk of infections that would preferentially elicit a Th1 response, such as viruses and other intracellular pathogens. In addition, free radicals produced during physical stress can impair the motility and functional capacity of neutrophils, which serve as a first line of defense against viruses.

The potential of vitamin C to sustain the functional capacity and motility of neutrophils via free radical scavenging and its actions in down-regulating the production of Th2 cytokines could mediate the reductions in the incidence of respiratory infections that have been reported in RCTs conducted among physically stressed populations. As mentioned previously for vitamin E, the antioxidant properties of vitamin C are likely to promote protection of cells in oxygen-rich environments (i.e., lungs) from excessive ROS, thus providing a mechanistic explanation for associations between supplementation and reduced severity and duration of respiratory infections. In addition, in vitro studies suggest that vitamin C inhibits viral replication, but this has not been confirmed in vivo. The potential benefits and risks of vitamin C supplementation at doses above 8000 mg/d on clinical outcomes have not been investigated. The potential role of vitamin C supplementation in non-respiratory infections has not been characterized in RCTs.

SUPPLEMENTATION WITH CAROTENOIDS

Carotenoids are a family of over 600 pigments from plants. About 50 of them, those with an unsubstituted β-ionone ring, possess vitamin A activity. Independent of their pro-vitamin A potential, carotenoids have a strong antioxidant capacity, mostly through direct quenching or modification of free radicals in oxidative reactions.[109,110] In human food sources, some of the most prevalent carotenoids include α-carotene, β-carotene, β-cryptoxanthin, lycopene, lutein, and zeaxanthin. The former three exhibit vitamin A activity after bioconversion in the body, and can be found abundantly in carrots and green leafy vegetables (β-carotene), and mandarin oranges (β-cryptoxanthin). Tomatoes are rich in lycopene, whereas lutein is found in watercress, parsley, and broccoli. This section reviews the effects of supplementation with carotenoids on parameters of immune function and their correlations with clinical outcomes. We also briefly summarize findings from supplementation studies with carotenoid-rich foods. We do not discuss supplementation with preformed vitamin A, because an extensive review of vitamin A supplementation, immune

function, and clinical outcomes in humans has been recently published.[6]

Carotenoid Supplementation and Innate Immunity

Carotenoids effectively quench free radicals, a function that could protect immune cells from the damaging effects of ROS generated by the cellular respiratory burst. In the next section, we discuss findings from studies examining the effects of carotenoid supplementation on parameters of the innate immune system, including circulating counts and functions of neutrophils, monocytes, and NK cells, and soluble mediators including circulating cytokines, salivary IgA, and immune components of breast milk.

Distribution of Cell Types

In a small (N = 7), non-controlled pilot study, no changes in the concentrations of circulating leukocytes were observed among HIV-positive men who were supplemented with 60 mg/d β-carotene for 4 weeks compared with baseline values.[111] All participants received micronutrients and maintained their antiretroviral therapy during the course of the supplementation period. In an RCT conducted in HIV-positive men and women from the United States (N = 21), supplementation with 180 mg/d β-carotene for 4 weeks significantly increased total leukocyte counts from baseline values compared with the placebo group[112] (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/). In a second study by the same research team,[113] 72 HIV-positive men and women were assigned to receive either 180 mg/d β-carotene or placebo for 3 months. No statistically significant differences between the supplemented and placebo arms were observed.

In an RCT of healthy smokers, supplementation with 40 mg/d β-carotene for 6 weeks was associated with significantly higher leukocyte counts in the supplemented group compared with the placebo group 6 weeks after supplementation ended, but not during the supplementation period (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/).[27] In nonsmoking older men and women from Ireland, low-dose carotenoid supplementation for 3 months (8.2 mg/d β-carotene or 13.3 mg/d lycopene) was not associated with changes in total leukocytes counts compared with placebo[114] (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/). The participants were not followed after the end of the supplementation period.

In non-controlled trials of β-carotene supplementation, the proportion of NK cells was significantly increased from baseline after supplementation in patients with oral leukoplakia (N = 16; 40 mg/d for 6 months),[115] but not in HIV-positive patients (N = 11; 60 mg/d for 4 months)[116] or in healthy adult women (N = 9; 15 mg/d for 28 days).[117] RCTs have shown little effect of supplementation with β-carotene on the proportion or absolute number of circulating NK cells in several populations (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/), including elderly men and women (N = 52, 8.2 mg/d for 12 weeks[114]; N = 54, 50 mg/d for 10–12 years[118]), adult smokers (N = 60, 40 mg/d for 6 weeks[27]; N = 45, 20 mg/d for 14 weeks[119]) and non-smokers (N = 50, 15–300 mg/d for 1 month[120]; N = 20, 60 mg/d for 44 weeks[121]), and HIV-positive persons (N = 72, 180 mg/d for 3 months[113]). One exception was a study among healthy, older adults who received ≥ 30 mg/d β-carotene for 2 months.[122] Supplementation was associated with significantly increased proportions of NK cells compared with placebo; however, 2 months after cessation of supplementation, the proportions of circulating NK cells had returned to baseline values. Only one RCT examined the effects of carotenoid supplementation on neutrophil counts. In the previously described US study in HIV-positive men and women,[113] supplementation with 180 mg/d β-carotene for 3 months did not alter counts of circulating neutrophils.

Monocytes do not appear to be influenced by carotenoid supplementation. In a non-controlled study among healthy adult women, counts of circulating peripheral monocytes were unaltered by β-carotene supplementation.[117] In the previously described RCT of Irish elderly,[114] neither low-dose lycopene nor β-carotene supplementation was associated with monocyte counts compared with the placebo group.

Cell Function

In vitro, ex vivo, and animal studies suggest that supplementation with carotenoids may enhance the functional capacity of NK cells[4] and preserve phagocytic cells from ROS by either suppression of respiratory burst[123] or quenching of radical oxygen species.[124,125] In a study of patients with oral leukoplakia (N = 16) the authors noted that, compared with baseline, NK cell cytotoxicity was significantly increased after 2 months of supplementation with β-carotene (30 mg/d); however, there was not a control group.[115] Findings from a long-term RCT support a role for β-carotene in enhancement of NK cell function in elderly, but not younger men (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/).[126,127] Middle-aged and elderly male participants in the Physicians' Health Study were randomized to receive either 50 mg of β-carotene every other day or placebo for 10 to 12 years. NK cell activity was assessed at the end of the study in a subgroup of 59

randomly selected participants. Among elderly participants, NK cell activity was significantly greater in those supplemented with β-carotene for 10 to 12 years compared with those who were randomized to the placebo group. A similar effect of supplementation was not observed in younger participants. Differences in NK cell activity were not due to increased production of stimulatory cytokines such as IL-2,[126] IL-12, IFN-γ, or IFN-α[127] by activated T-cells. The authors suggested that β-carotene may have upregulated signaling events that are important to the lytic cycle or the production of other stimulatory mediators such as TNF-α or IL-15.[126]

Animal studies indicate that carotenoids may suppress the respiratory burst in phagocytic cells.[123] In a depletion-repletion study, 10 nonsmoking older men adhered to a 3-week carotenoid depletion diet followed by supplementation with mixed carotenoids for 5 weeks (30 mg/d β-carotene, 15 mg/d lycopene, 9 mg/d lutein).[125] Hydrogen peroxide production by PMA-stimulated neutrophils was significantly increased at the end of the depletion period compared with baseline. Supplementation restored hydrogen peroxide production to baseline values. However, the results can not be causally attributed to β-carotene, because control groups were not involved. In a randomized, controlled depletion-repletion study, young adult, nonsmoking males (N = 15; 18–30 years of age) were placed on a carotenoid-free liquid experimental diet for 2 weeks[128] (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/). After the 2-week depletion period, subjects were randomized to receive liquid diets supplemented with either 15 mg/d or 120 mg/d β-carotene for 4 weeks. No differences in ROS production by stimulated neutrophils were observed between the two supplement arms. Superoxide production by neutrophils did not change after 4 weeks of supplementation with either dose compared with values obtained immediately following the 2-week depletion diet. In an RCT of healthy smokers, supplementation with β-carotene (40 mg/d for 6 weeks) resulted in significantly reduced generation of ROS by mitogen-stimulated phagocytes after 4 and 6 weeks of supplementation.[27] Similar reductions were not observed in those receiving placebo. Estimates of differences in responses between the placebo and supplemented groups were not reported.

The expression of adhesion molecules on the surface of cells of the innate immune system is important in the transition from an innate to an adaptive response. Randomized, controlled crossover trials in healthy male nonsmokers support a role for carotenoid supplementation on the expression of adhesion markers.[129] In one RCT, β-carotene supplementation (15 mg/d for 26 days) was associated with increased percentage of monocytes expressing HLA-DR, HLA-DP, ICAM-1, LFA-1, and LFA-3.[129] Significant increases in the relative number of ICAM-1 and LFA-3 molecules, but not MHC class II molecules, on monocyte surfaces were also noted with β-carotene supplementation. In a separate RCT, supplementation with lycopene (15 mg/d for 26 days) also significantly increased the percentage of monocytes expressing HLA-DR and the absolute number of LFA-1 molecules compared with placebo. Lutein supplementation (15 mg/d for 26 days) decreased expression of HLA-DQ compared with baseline values.[130]

Soluble Mediators

A randomized, placebo-RCT in Bangladeshi women examined the effects of supplementation with a single vitamin A dose, daily β-carotene, or placebo from 1 to 2 weeks postpartum to 9 months postpartum on breast milk immune factors (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/).[131] Supplementation with β-carotene was not associated with breast milk concentrations of sIgA, lactoferrin, lysozyme, or IL-8 compared with either vitamin A or placebo. In the previously described crossover study of healthy, nonsmoking men, supplementation with 15 mg/d β-carotene for 26 days was associated with significantly greater increases from baseline in TNF-α secretion by blood monocytes when cultured ex vivo (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/).[129]

In a randomized pilot study, healthy Finnish men were supplemented with either 20 mg/d β-carotene or placebo for 60 days to assess the effects of supplementation on skin discoloration.[132] At the end of the supplementation period, a subsample of participants (n = 89) participated in post-supplementation analyses of salivary proteins. Concentrations of sIgA did not differ between the two treatment arms; data on pre-supplementation concentrations of sIgA were not collected.

Carotenoid Supplementation and Adaptive Immunity

Several non-controlled and randomized controlled studies have examined the effects of β-carotene supplementation on parameters of the adaptive immune response. In the next section we review findings related to lymphocyte counts, lymphocyte proliferation, and production of cytokines, the DTH response, and measures of humoral immunity.

Lymphocyte Subpopulations

Multiple studies have examined the effects of β-carotene supplementation on the distribution of lymphocytes in healthy populations. An early non-controlled study in healthy adult males (N = 17)[133] reported in-

creased CD4 cell counts from baseline after 2 weeks of supplementation with 180 mg/d β-carotene. Additional non-controlled studies have not reported an effect of carotenoid supplementation on lymphocyte subpopulations.[115,117,133]

Only one RCT, conducted in healthy older men and women (N = 20), has reported an effect of β-carotene supplementation (30–60 mg/d) on lymphocyte counts; CD4 cell counts increased from baseline after 2 months of supplementation (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/).[122] Other RCTs did not find significant effects of carotenoid supplementation at doses ranging from 15 to 300 mg on the proportions or absolute counts of circulating total T-cells,[114,118-121] CD3,[118,119,122,126] CD4,[114,118-121,126] or CD8 cells,[114,118-120,122,126] cytotoxic T-cells,[119,121] or memory T-cells.[119,121] Of the four RCTs that have examined changes in the CD4/CD8 ratio,[114,118,121] only one observed a statistically significant effect of carotenoid supplementation[121] (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/). The study in elderly Irish persons[114] used a lower dose of β-carotene (8.2 mg/d) compared with the other three studies (50 mg/d,[118] 90 mg/d,[118] and 60 mg/d[121]), while the 10- to 12-year supplementation study in older men[118] did not have data available on lymphocyte counts at baseline. The other two studies, although similar in dose and duration, differed in the age and gender distributions of their study populations and their findings may not be directly comparable.

The effects of carotenoid supplementation on lymphocyte subsets in populations with chronic illnesses have also been reported. In a small, non-controlled study of patients with oral leukoplakia (N = 16), supplementation with 30 mg/d β-carotene for 6 months had no significant effects on CD3, CD4, or CD8 cell counts compared with baseline values.[115] A study among AIDS patients noted a non-statistically significant 66% increase in total lymphocyte counts from baseline values after 4 weeks of β-carotene supplementation.[111] After excluding three subjects with CD4 cell counts over 10 /μL, CD4 cell counts were increased significantly from baseline (43%, $P < 0.05$). No changes in CD8 cell counts or the CD4/CD8 ratio were observed. All patients were on antiretroviral therapy and all were receiving a micronutrient supplement.[111] In a separate non-controlled study of HIV-positive patients who had not progressed to AIDS (N = 11), supplementation with 60 mg/d β-carotene for 4 months was associated with a significantly reduced proportion of CD11 and CD8 cells 6 months after initiation of supplementation (2 months after cessation of supplementation) compared with both baseline and values at 3 months post-supplementation.[116] No changes were observed with respect to the proportion of

CD4, IL-2 receptor-expressing T-cells, or the number of total lymphocytes.

Some RCTs in populations with chronic illnesses have also reported on the effects of carotenoid supplementation. β-Carotene supplements (30 mg/d for 3 months) given to colon cancer patients (n = 18) and patients with colonic polyps (n = 19) were associated with significant increases in the number of circulating CD4 cells and IL-2 receptor-expressing T-cells compared with baseline values.[134] Similar enhancements were not observed in the placebo group. Supplementation was not associated with changes in the distributions of CD8 cells. In a non-placebo-randomized, controlled study, HIV-positive patients with CD4 counts under 400/μL were supplemented with either β-carotene (60 mg/d; N = 15) or selenium (250 μg/d; N = 15) for 1 year.[135] An additional group of non-supplemented HIV-positive patients (N = 22) served as the control group. No significant changes from baseline or differences between groups were reported over the course of the study with respect to CD4 cell counts (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/). In a small placebo-RCT among HIV-positive men and women (N = 21), supplementation with 180 mg/d β-carotene for 4 weeks was associated with significantly greater increases in CD4 cell counts and the CD4/CD8 ratio from baseline compared with placebo.[112] However, a larger study (N = 72) conducted by the same group reported no differences in the circulating levels of total lymphocytes, CD4 cells, CD8 cells, or CD3 cells between treatment arms at any point during the study.[113] Although the supplement dose was similar in these two studies, other differences, such as duration of supplementation, receipt of micronutrient supplements, differences in use of drug therapy, disease stage, compliance, and dropout rates may have contributed to the inconsistent findings. In a recent, placebo-RCT,[331] adults with advanced AIDS were randomized to two groups; daily supplementation with either vitamin A and trace elements or supplementation with vitamin A, trace elements, and mixed carotenoids (68.4 mg/d β-carotene, 2.2 mg/d α-carotene, 0.4 mg/d zeaxanthin, 0.5 mg/d cryptoxanthin, 0.4 mg/d lutein) for an average of 13 months.[136] CD4 counts in the group who received carotenoids were significantly higher at 12, 15, and 18 months compared with those not supplemented.[136]

Lymphocyte Proliferation

Non-controlled[116,117] and RCTs (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/)[114,118,120,137] in nonsmoking adult and elderly populations suggest that β-carotene supplementation does not influence resting or mitogen-induced lymphocyte proliferation. In a random-

ized, placebo-RCT of male cigarette smokers supplemented for 14 d with 20 mg/d β-carotene, PHA- stimulated lymphocyte proliferation in the supplemented group was significantly higher after 14 weeks compared with placebo.[119] Lymphocyte proliferation in response to PHA + fetal calf serum or ConA was not affected by supplementation. In the other RCTs among smokers,[27] the effects of β-carotene supplementation on lymphocyte proliferation were not examined.

Mediators of Adaptive Immunity

Placebo-RCTs have shown no impact of supplementation with β-carotene (8.2–300 mg/d) on production of IL-2[114,118,120,126] or IL-4[114] by PBML in healthy adult or elderly populations. Similarly, several RCTs have indicated that neither short-term[118] nor long-term supplementation (10–12 years) with β-carotene had effects on lymphocyte production of PGE2 in healthy, nonsmoking middle aged men,[126] elderly men,[118,126] or elderly women[118] (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/).

Delayed-Type Hypersensitivity Response

In two placebo-RCTs, supplementation with 30 mg/d β-carotene prevented UV-induced suppression of DTH responses as measured by the number of positive tests and the cumulative diameter of indurations in both healthy adult[138] and older men[139] (Table 3; please go to www.ilsi.org/Publications/NutritionReviews/).

Humoral Immunity

No effect of carotenoid supplementation has been observed on counts of circulating B-cells.[114,117-121] To date, no studies have examined the effects of carotenoid supplementation on circulating levels of serum immunoglobulins or vaccine response.

Carotenoid Supplementation and Clinical Outcomes

Respiratory Infections

One RCT evaluated the effects of carotenoid supplementation on respiratory illness among male smokers (N = 21,796; 50–69 years of age) who participated in the Alpha-Tocopherol Beta-Carotene Cancer Prevention study of Finland.[52] Compared with placebo, β-carotene supplementation (20 mg/d) for 6 years was not associated with the incidence of common colds[54] or the first occurrence of hospital-treated

pneumonia.[55] In a subset of participants who engaged in heavy exercise, supplementation with β-carotene appeared to increase the incidence of common colds.[53] In addition, in men who initiated smoking later in life, β-carotene supplementation was associated with a 40% increase in the risk of a first occurrence of hospital-treated pneumonia.[55] It has been suggested that conditions that promote an oxidative environment, such as strenuous exercise and smoking, may trigger the production of excessive carotenoid cleavage products that can have pro-oxidant activity.[124] Because carotenoid cleavage products have been observed to stimulate ROS production by PMA-activated neutrophils,[140] it is plausible that increased ROS production, potentially elicited by β-carotene cleavage by-products, promoted lung tissue damage in these groups. ROS-associated damage to the airways could render the respiratory system more susceptible to viral infections or unable to prevent progression to a more severe stage of illness. The generalizability of these findings to nonsmoking populations and to women is uncertain.

HIV/AIDS

As described earlier, the evidence regarding the effects of supplementation of HIV/AIDS patients with carotenoids on the distribution and functions of leukocyte and lymphocyte subsets is not conclusive. Findings from several prospective studies indicate that low plasma concentrations of carotenoids may predict disease progression and contribute to increased mortality, while higher intakes from diet or supplements may reduce morbidity and enhance survival.[141-144] However, a number of RCTs have included preformed vitamin A in the supplementation regimen,[145-149] and it is not possible to attribute the effects to carotenoids alone. In the RCT of adults with advanced AIDS,[136] multivariate analyses suggested that survival was significantly improved in those with higher serum β-carotene concentrations at baseline irrespective of treatment group. Additionally, after adjusting for prognostic indicators, the subjects who received carotenoids had a 3-fold lower risk of death compared with those receiving the vitamin A and trace elements without carotenoids. No differences in the two treatment arms were observed with respect to changes in viral load, the risk of new or recurrent AIDS-associated illnesses, or risk of hospitalization for reasons unrelated to AIDS.[136]

Other Infectious Illnesses

Some observational studies have shown that carotenoid concentrations are depressed in children with diarrhea[150] or malaria[151-154] compared with uninfected children. The effects of supplementation with carote-

noids on diarrhea or malaria outcomes have not been examined in RCTs.

Carotenoid-Rich Foods and Parameters of Immune Function

Several RCTs have assessed the role of carotenoid rich foods in influencing parameters of both the innate and adaptive arms of the immune response.

Innate Immunity

In a non-placebo-controlled, crossover trial, adult men (N = 22) were randomized to receive supplementation with either carrot juice (27.1 mg/d β-carotene, 13.1 α-carotene) or tomato juice (37 mg/d lycopene) following an initial 14-day carotenoid-depletion period.[155] After 14 days of juice supplementation, subjects adhered to a 2-week low-carotenoid washout diet and then crossed over to the other juice for 14 days. The second 14-day supplementation period was followed by a 3-week low-carotenoid diet. Subjects abstained from carotenoid-rich foods for the duration of the study. Significant increases in NK cell lytic activity were observed for both juices compared with baseline values.[155] There was a 2-week lag period from the time increases in serum concentrations of the carotenoids were observed to the time when changes in immune parameters occurred; however, comparisons could not be made against a placebo group. In an RCT of smoking and nonsmoking males (N = 55) who consumed an experimentally designed carotenoid-deficient diet, supplementation with tomato extract for 2 weeks (4.88 mg/d lycopene; 0.48 mg/d phytoene; 0.44 mg/d phytofluene; 1.18 mg/d α-tocopherol) was not associated with changes in NK cell lytic activity compared with a placebo group, and no differences were observed by smoking status.[156] In both of these studies, a carotenoid-only group was not included, thus it is difficult to attribute findings to a carotenoid-specific effect.

Adaptive Immunity

Two studies have evaluated the effect of supplementation with carotenoid-rich foods on lymphocyte proliferation. In the previously described carrot and tomato juice-crossover study,[155] ConA-induced lymphocyte proliferation decreased significantly from baseline during the initial carotenoid depletion period. Proliferation was restored by both carrot and tomato juice 2 weeks after the first intervention period ended. Lymphocyte proliferation was maintained for the duration of the study at a level significantly higher than the levels observed at the end of the initial depletion period. In the tomato

extract study described above,[156] supplementation had no effects on lymphocyte proliferation in either smoking or nonsmoking males. However, the dose of lycopene obtained from the daily supplement (4.88 mg/d) was lower than that used in the carrot and tomato juice study (37 mg/d), and additional measures were not obtained after supplementation ended.

In the carrot and tomato juice crossover study, production of IL-2 and TNF-α by mitogen-stimulated lymphocytes was increased after the administration of each juice,[155] whereas mitogen-induced IL-4 production was not altered. In a separate crossover study, tomato juice (40 mg/d lycopene), carrot juice (22.3 mg/d β-carotene), and dried spinach (11.3 mg/d lutein) were administered sequentially for 2 weeks after an initial 2-week depletion period.[157] Interleukin-2 and IL-4 production by stimulated lymphocytes significantly decreased during the 2-week depletion diet but were restored to baseline levels after the first 2-week period of tomato juice. Production of both IL-2 and IL-4 declined with the administration of carrot juice and spinach powder to levels that were similar to those observed immediately following the 2-week depletion period. In contrast, in the study of smoking and nonsmoking males who consumed an experimentally designed carotenoid-deficient diet, supplementation with tomato extract did not alter lymphocyte production of IL-2 or TNF-α compared with a placebo group.[156] IL-4 production was significantly reduced after 2 weeks of supplementation among smokers such that post-supplementation levels were reduced to levels similar to those of nonsmokers; a similar decline in IL-4 production was not seen among smokers in the placebo group.[156] In a non-controlled study, supplementation with a lycopene-rich tomato drink (47.1 mg/d lycopene) for 8 weeks did not alter the DTH response in healthy elderly men and women compared with baseline values.[158]

Findings from studies that have utilized carotenoid-rich, food-based supplements should be cautiously interpreted with respect to the effect of carotenoids on immune parameters. The studies described did not use a placebo or a pure carotenoid group for comparisons. It is plausible that additional, unmeasured constituents in the carotenoid-rich supplements were responsible for the observed changes on immune parameters. To date, no clinical trials have been conducted to examine the effects of supplementation with carotenoid-rich foods on clinical outcomes.

Carotenoid Supplementation Summary

In contrast to supplementation trials with vitamins C and E, RCTs of carotenoid supplementation and immune function have been conducted in populations that are largely generalizable to healthy adults and the elderly.

These studies suggest that carotenoid supplementation has little influence on the distributions or functional capacity of innate immune cells in healthy nonsmokers. Supplementation with β-carotene appeared to increase counts of total circulating leukocytes among HIV-positive men and women and in smokers; however, the clinical significance of the observed increase is unclear. RCTs have demonstrated that supplementation could enhance NK cell activity in the elderly and reduce the production of ROS by neutrophils in smokers.

With respect to adaptive immunity, carotenoid supplementation does not appear to influence immune parameters in healthy, nonsmoking adult populations. However, supplementation was associated with enhanced expression of several markers of cell adhesion, which could suggest that β-carotene may facilitate the activation of adaptive immune cells. Supplementation improved CD4 counts in populations with chronic illnesses such as cancer and HIV, and enhanced mitogen-stimulated lymphocyte proliferation in smokers. β-carotene supplementation also preserved the DTH response during immunosuppressive UV exposure.

While carotenoid-rich foods appear to influence aspects of both innate and adaptive immunity, the possibility that these effects are due to other active components in the foods cannot be ruled out. The role of carotenoids or carotenoid-rich foods in aspects of humoral immunity is uncertain.

An effect of supplementation with preformed vitamin A on immune function is well documented, and it is plausible that certain immunomodulatory effects observed with carotenoid supplementation could be a result of conversion to vitamin A. However, several studies noted that carotenoid supplementation did not alter vitamin A status, indicating that carotenoids were not being used only as precursors to vitamin A. To our knowledge, no RCTs have assessed the effects of supplementation on vaccine response. Trials investigating the effects of supplementation on barrier function are limited. Also, few studies have evaluated the immunological effects of carotenoids other than β-carotene, such as those without pro-vitamin A activity.

RCTs of carotenoids and respiratory illness are limited to one study in older, smoking men, and the findings suggest that supplementation may increase morbidity in subjects who initiated smoking later in life or who engaged in strenuous exercise. Additional RCTs in women, non-smokers, and athletes would be useful in clarifying the impact of carotenoid supplementation on respiratory illnesses. One RCT in HIV-positive persons suggested that supplementation with β-carotene may decrease disease progression and promote survival, possibly via maintenance of CD4 cell counts. Other studies have been less conclusive regarding an independent role for carotenoid supplementation in HIV-related outcomes due to the inclusion of vitamin A or other nutrients in the supplement preparation. We are unaware of any RCTs that have examined the effects of carotenoid supplementation on immune function or clinical outcomes in children. However, a limited number of observational studies suggest a potential role for carotenoids on diarrhea and malaria outcomes in children; these findings warrant further investigation.

SUPPLEMENTATION WITH VITAMINS IN COMBINATION

In the next sections, we discuss the immunological effects of supplementation with antioxidants in combination and B-vitamin containing multivitamins, since RCTs of single B vitamins are lacking. We do not discuss studies that included minerals as part of the supplement in this review.

Vitamins in Combination and Innate Immunity

Distribution of Cell Types

In a controlled crossover study, endurance athletes (N = 12) were randomized to receive either an antioxidant supplement containing 18 mg/d β-carotene, 900 mg/d vitamin C, and 90 mg/d vitamin E or placebo for 7 days prior to an intense exercise test (2-hour treadmill run at 65% VO2max; Table 4; please go to www.ilsi.org/Publications/NutritionReviews/).[159] Subjects then completed a 2-week washout prior to crossing over to the other treatment arm for the second 7-day supplementation period and subsequent exercise trial. The supplements had no effect on the patterns of change in total leukocyte or neutrophil counts.[159] In an RCT of male runners from Denmark (N = 20), supplementation with 500 mg/d vitamin C and 400 mg/d vitamin E for 14 days prior to and 7 days after a treadmill run did not attenuate exercise-induced increases in neutrophils, monocytes, or NK cells compared with placebo.[160] In a long-term, 2×2 factorial design RCT in France, 756 institutionalized elderly men and women from multiple geriatric centers were randomized to one of four treatment groups: 1) antioxidant vitamins only (6 mg/d β-carotene, 120 mg/d vitamin C, 15 mg/d vitamin E); 2) antioxidant vitamins + trace minerals; 3) trace minerals only; or 4) placebo.[161,162] Counts of circulating immune cells were analyzed in a subset of the participants (N = 134) at baseline and 6 months after supplementation began.[161] The antioxidant vitamins did not have an effect on the proportion of circulating NK cells (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/).

Cell Function

The majority of studies examining the effects of antioxidant supplements on the functional capacity of innate immune cells have focused on neutrophils. In a non-controlled study of healthy (N = 10) and ill (N = 20, 10 with major depressive disorder and 10 with coronary heart disease) elderly women, supplementation with 1000 mg/d vitamin C in combination with 200 mg/d vitamin E for 16 weeks increased the phagocytic functions of neutrophils.[163]

Randomized trials evaluating the influence of antioxidant supplementation on neutrophil function have been conducted in athletes. In the previously described study of male runners (N = 12),[159] the respiratory burst of neutrophils was increased post-exercise and this increase was significantly greater in the antioxidant-supplemented athletes compared with the placebo group. In a second study, endurance athletes were randomized to receive either placebo or an antioxidant supplement of β-carotene (30 mg/d) and vitamins C (1000 mg/d) and E (500 mg/d) while maintaining their normal training schedule[164] (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/). Supplementation for 3 months was associated with significantly higher activities of the neutrophil antioxidant enzymes catalase, glutathione reductase, and SOD compared with placebo. Similarly, concentrations of total glutathione and the GSH/GSSG ratio were significantly higher at 3 months compared with baseline in the supplemented group. No changes from baseline were observed in the placebo group. Taken together, these two studies suggest that antioxidant supplementation may enhance the functional capacity of neutrophils during extreme exercise that potentially increases ROS production. However, supplementation may also promote an upregulation of antioxidant systems, which may serve to counteract increases in ROS production and protect neutrophils from ROS-induced damage.

Soluble Mediators

Exercise-induced increases in inflammatory cytokines may be a function of the stimulatory effects of free radicals on cytokine production. The ability of antioxidants to scavenge free radicals may indirectly influence innate immunity by attenuating cytokine production.[160] The effects of supplementation with a combination of vitamins A, C, and E on cytokine production by monocytes were assessed in a non-randomized clinical trial. Athletes who were not exercising for 1 month prior to the initiation of supplementation were supplemented for 60 days with a combination of 50,000 IU/d vitamin A, 1000 mg/d vitamin C, and 200 mg/d vitamin E.[165] An exercise test was conducted prior to and post-supplementation. Serum levels of TNF-α, IL-6, and IL-1β and production of these cytokines by LPS-stimulated monocytes was measured after each exercise test. Supplementation significantly reduced exercise-induced increases in serum concentrations of TNF-α, IL-6, and IL-1β, and these reductions were not a result of reduced cytokine production by monocytes. Lack of randomization, the inclusion of vitamin A in the supplement, and the small sample size (N = 6) limit interpretations regarding a causal effect of antioxidants.

In the RCT of athletes exposed to an exercise challenge,[160] supplementation with vitamins C and E resulted in a nonstatistically significant attenuation of exercise-induced increases in IL-1RA concentrations compared with placebo (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/). In an additional RCT, physically active nonathletes (N = 14) were randomized to receive either supplementation with vitamins C (500 mg/d) and E (268 mg/d) or a placebo for 29 days prior to a 3-hour exercise challenge.[166] Although supplementation had no effects on IL-1RA, exercise-induced increases in plasma IL-6 concentrations were 50% lower in the antioxidant-supplemented group. The net production of IL-6 by working muscles was approximately 6-fold higher in the placebo compared with the supplemented group. These findings suggest that antioxidant supplementation could influence the immune function via reductions in IL-6 production by exercising muscles.[166] In the multicenter study of elderly French patients, those supplemented with antioxidants had significantly greater IL-1 production by LPS-stimulated monocytes 6 months after initiation of supplementation compared with the placebo group.[161]

Studies with B-Containing Multivitamins

One RCT examined the effect of vitamin B_6 supplementation on parameters of innate immunity in hospitalized Taiwanese men and women.[167] Fifty-one patients who were admitted to the intensive care unit were randomized to one of three groups: 1) 100 mg/d B_6 injection; 2) 50 mg/d B_6 injection; or 3) control (no injection). Supplementation began within 24 hours of ICU admission and continued for 14 days. No statistically significant differences were observed at day 14 for neutrophil counts in any group compared with baseline values; however, the percentage of neutrophils was significantly lower than the baseline value in the control group at day 14 (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/). RCTs evaluating the effect of supplementation with B-containing multivitamins on immune function have focused on parameters of adaptive immunity; these will be considered in the next section, along with studies of antioxidant combinations.

Vitamins in Combination and Adaptive Immunity

Lymphocyte Subpopulations and Proliferation

Among 30 elderly hospitalized men in the United Kingdom, supplementation with a combination of vitamins A (8000 IU/d), C (100 mg/d), and E (50 mg/d) for 28 days resulted in significantly greater increases in the CD4/CD8 ratio[168] (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/; $P < 0.05$). There were marginally significant effects of treatment on the absolute counts of circulating lymphocytes, total T-cells, CD4 cells, and CD8 cells compared with baseline values. No significant changes were observed in the placebo group. PHA-induced lymphocyte proliferation was similarly increased from baseline in the supplemented group but not in the placebo group. However, because the supplement contained vitamin A, findings may not be attributable to the effects of vitamins C or E.[168]

In the RCT among Danish runners, antioxidant supplementation for 14 days had no effect on lymphocyte counts, including CD4, CD8, and CD3 subsets[160] (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/). Similarly, daily supplementation in the multicenter French study of elderly subjects did not alter the proportion of CD3, CD4, or CD8 cells, the CD4/CD8 ratio, or lymphocyte proliferation.[161]

The majority of studies investigating the effects of supplementation with B vitamins or B-containing multivitamins have been conducted in immunocompromised or elderly populations. In the Taiwanese study described previously, supplementation of ICU patients with 50 mg/d or 100 mg/d of vitamin B_6 was associated with significant increases in the number of total circulating lymphocytes, CD3 cells, CD4 cells, and CD8 cells after 2 weeks compared with a control group.[167] There were no effects on B cells. In an RCT of Japanese gastric cancer patients, supplementation with a vitamin B-complex (107 mg/d B_1, 100 mg/d B_6, 1 mg/d B_{12} in 500 mL of glucose solution) for 14 days following gastric resection surgery was associated with significantly attenuated post-surgery depressions of PHA-stimulated lymphocyte proliferation compared with the control group.[169] Four weeks after surgery (2 weeks after cessation of supplementation), a significantly greater number of patients in the supplemented group had restored lymphocyte proliferation to pre-surgery levels compared with the control group.

In a 2×2 factorial RCT (N = 1078), pregnant HIV-positive women from Tanzania were randomized to one of four treatment arms: 1) daily multivitamin (20 mg B_1, 20 mg B_2, 25 mg B_6, 100 mg niacin, 50 μg B_{12}, 500 mg vitamin C, 30 mg vitamin E, 0.8 mg folate); 2) vitamin A/β-carotene only (vitamin A 5000 IU, β-carotene 30 mg); 3) multivitamins + vitamin A/β-carotene; or 4) placebo. Supplementation was initiated in pregnancy and continued through lactation to the end of the study (median duration, 71 months).[170] Blood samples were collected at baseline and every 6 months thereafter for analysis of lymphocyte subsets. Multivitamin supplementation was associated with significant increases in mean CD4, CD8, and CD3 cells counts over the course of follow-up[147] (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/). In addition, multivitamin supplementation of mothers during pregnancy and lactation significantly increased CD4 cell counts in their infants at 6 and 24 months of age compared with placebo.[171] Other RCTs have examined the effects of micronutrient supplementation in HIV-positive populations on lymphocyte counts. Three studies reported increased CD4 counts with supplementation,[172-174] while one study in Thailand reported no effect.[175] However, these studies included trace minerals or other nutrients as part of the supplement, confounding interpretations for a potential effect of vitamins.

Mediators of Adaptive Immunity

In the multicenter French study, production of PHA-stimulated IL-2 cytokines by PBMLs isolated from elderly men and women was not influenced by supplementation with antioxidants for 1 year[161] (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/).

Delayed Type Hypersensitivity Response

In a non-controlled study of Croatian men and women living in elderly care homes (N = 72; 60–89 years of age), supplementation with multivitamins (15 mg/d B_1, 15 mg/d B_2, 50 mg/d B_3, 10 mg/d B_6, 10 μg/d B_{12}, 25 mg/d pantothenate, 7.5 mg/d β-carotene, 250 mg/d vitamin C, and 200 mg/d vitamin E) appeared to reverse age-associated decreases in the DTH response.[176] Several RCTs have examined the effects of supplementation with vitamins in combination on the DTH response. In a therapeutic trial in India, 174 children 2 to 35 months of age who had been admitted to a hospital with acute lower respiratory infection were randomized to receive either a combination of vitamins C (100 mg) and E (200 mg) or a placebo twice daily for 5 days[177] (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/). The DTH response to seven antigens was assessed on admission and 2 weeks later. Supplementation did not influence the DTH response. In the multicenter RCT of institutionalized elderly men and women in France, supplementation with antioxidant vitamins had no effect on the DTH response.[161] Similarly, in a study by the same researchers in which a similar

design and supplementation regimen were implemented, antioxidants did not have an effect on the DTH response.[162] The doses used in single-nutrient studies of DTH response were nearly 10-fold greater than those administered in the French studies; this could partly explain why the latter were null. In the RCT of Japanese cancer patients,[169] supplementation with a vitamin B-complex immediately following gastric resection significantly attenuated surgery-related depressions in the DTH response to tuberculin. The response to other antigens was not examined. Additional studies of B-vitamin-containing multivitamins have not examined the DTH response.

One study evaluated the effects of antioxidants in combination on measures of humoral immunity. In the multicenter study of elderly persons in France,[162] comparisons between the placebo group and the group supplemented with antioxidant vitamins suggested that the antibody response to influenza vaccine was negatively influenced by supplementation with combined antioxidants. The proportions of sero-protected subjects were lower in the supplemented group at each time point measured compared with placebo. At the end of the follow-up period, the proportion of sero-protected subjects in the multivitamin arm was approximately half that in the placebo group (Table 4; please go to www.ilsi.org/Publications/NutritionReviews/).[162] It is unclear why supplementation with antioxidants would decrease the response to influenza vaccine. While reductions in vaccine response to several pathogens were not observed in the previously discussed studies of vitamin E supplementation alone, neither the effect of vitamin E on influenza vaccine nor the effects of vitamin C or β-carotene on vaccine response to any pathogens have been examined. The US Centers for Disease Control currently recommends that all persons older than 65 years be inoculated against influenza. Older populations are at increased risk of nutritional deficiencies due to physiological metabolic changes that occur from aging and may benefit from vitamin supplementation.[178,179] The participants in the French study had low mean serum β-carotene and vitamin C concentrations at baseline (approximately 457 and 3.3 μg/mL, respectively) that were corrected with supplementation.[161] Additional RCTs are warranted to clarify the effects of supplementation with antioxidants, singly and in combination, on vaccine response in older populations.

Vitamins in Combination and Clinical Outcomes

HIV/AIDS

HIV-positive patients (N = 49) from Canada were randomized to receive daily either a placebo or a com-bination supplement of vitamin C (1000 mg) and vitamin E (360 mg) for 12 weeks.[180] A trend toward reduced viral load and decreased oxidative stress was noted in those supplemented with vitamin C and vitamin E compared with placebo. In the study of HIV-positive Tanzanian women, supplementation with multivitamins significantly reduced viral load, delayed disease progression, and lowered mortality due to AIDS-related complications compared with the placebo group.[147] Multivitamins also reduced the risk of wasting, measured as the first incident of mid-upper-arm circumference under 22 cm[181] and increased weight gain in pregnancy.[182] Prenatal and postnatal supplementation of HIV-positive mothers with this combination of multivitamins appeared to reduce the transmission of HIV via breast-feeding in infants born to women who were the sickest.[183] The benefits of multivitamin supplementation to the mothers extended to their children, and included lowered risks of acute, watery, and all-cause diarrhea,[184] a trend towards reduced child mortality,[185] and improved growth.[186]

Additional studies of supplementation with vitamins in combination and HIV-related outcomes have included trace minerals or other nutrients as part of the supplementation regimen,[172,173,175,187] and are not included in this review of vitamins only.

Other Infections

Both the multicenter and single-center RCTs in institutionalized elderly persons from France reported no effect of antioxidant vitamin supplementation on respiratory or urogenital infections.[162,188] In contrast, supplementation with single antioxidant vitamins has been associated with reduced incidence of respiratory morbidity in the elderly[189] and in populations undergoing physical stress.[95] These apparent discrepancies could be partly explained by the use of higher doses in the single-nutrient studies compared with the antioxidant combination trials. Future studies need also to consider potential interactions between antioxidant vitamins administered simultaneously. In the previously described prophylactic study in India, supplementation of children who had severe respiratory illness with vitamins C and E did not influence the duration of the illness or any indicators of severity such as fever, feeding difficulty, or tachypnea.[177] The lack of effect observed in this study is consistent with findings from studies of therapeutic vitamin C supplementation; the therapeutic use of vitamin E supplementation at the onset of infections has not been examined.

It is difficult to elucidate the role of vitamins in diarrhea and respiratory infections, because the majority of studies assessing the impact of micronutrient supplements also included trace minerals[190-192] or macronutrients.[193]

Combination Vitamin Supplementation Summary

The studies reviewed indicate that supplementation with combinations of antioxidants may modulate the functional capacity of neutrophils and cytokine production by monocytes. In athletes specifically, supplementation with combinations of antioxidants appeared to increase respiratory burst and upregulate the antioxidant systems of neutrophils, suggesting a potential to enhance protection of these cells from free radical damage. In addition, antioxidant supplementation was associated with reduced IL-6 production from exercising muscles. Together, these actions of antioxidants might attenuate exercise-associated immunosuppression. In controlled studies among elderly populations, antioxidants provided in combination did not appear to influence the number or function of cells of the innate or adaptive arms of the immune system. These findings would be consistent with findings from a single study noting no effect of supplementation with antioxidant vitamins on the incidence of respiratory or urogenital infections in elderly people. However, the vitamin doses in the combined antioxidant studies were lower than those used in the single antioxidant supplementation studies showing benefits on respiratory illness. One study of therapeutic antioxidant vitamin supplementation in children with acute lower respiratory infection did not demonstrate an effect on cell-mediated immunity or measures of severity or duration of respiratory illness. Other parameters of immunity have not been examined in children. Supplementation with combinations of antioxidants does not appear to influence cellular immunity as measured by the DTH response. One RCT in surgery patients, however, suggested that supplementation with vitamin B-complex may reduce trauma-associated depressions in DTH response.

Among HIV-positive patients, antioxidants reduced oxidative stress and viral load, whereas multivitamin supplementation increased lymphocyte counts. These improved immune parameters are likely to correlate with the observed benefits of multivitamins on HIV-associated clinical outcomes, including opportunistic infections, disease progression, and mortality. One RCT has examined the effects of micronutrient supplementation among HIV-positive patients who are receiving anti-retroviral therapy,[173] and found significant increases in lymphocyte counts; however, the supplement used in this study included trace minerals and other antioxidant nutrients (e.g., N-acetylcysteine) in addition to vitamins. Additional studies to examine the effects of multivitamin supplements in persons receiving anti-retroviral therapy are needed.

It is difficult to differentiate whether the observed effects of vitamins in combination are due to the actions of single nutrients or to synergistic effects between the nutrients. Most of the studies investigating the influence of antioxidant vitamins on specific parameters of immune function have largely been conducted in adult or elderly populations who were exposed to oxidative stress or were immunologically compromised. Few studies have been conducted in children or in populations residing in developing countries where the rates of malnutrition and subsequent immunosuppression and risk of infectious disease are high.

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