Effect of Long-term Dietary Antioxidant Supplementation on Influenza Virus Infection

Sung Nim Han,1 Mohsen Meydani,1 Dayong Wu,1 Bradley S. Bender,2 Donald E. Smith,1 José Viña,3 Guohua Cao,4 Ronald L. Prior,1 and Simin Nikbin Meydani1,5

1Jean Mayer Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts.
2Department of Medicine, University of Florida College of Medicine, and Geriatric Research, Education and Clinical Center, Malcolm Randall Veterans Affairs Medical Center, Gainesville, Florida.
3Departamento Fisología, Facultad de Medicina, University of Valencia, Spain.
4Nutritional Science Department, University of Connecticut, Storrs.
5Department of Pathology, Sackler Graduate School of Biomedical Sciences, Tufts University.

This study compared the effect of vitamin E on the course of influenza infection with that of other antioxidants. (In a previous study we showed that short-term vitamin E supplementation significantly decreased pulmonary viral titer in influenza-infected old mice). Eighteen-month-old C57BL/6NCrBR mice were fed one of the following semisynthetic diets for 6 months: control, vitamin E supplemented, glutathione supplemented, vitamin E and glutathione supplemented, melatonin supplemented, or strawberry extract supplemented. After influenza virus challenge, mice fed vitamin E-supplemented diet had significantly lower pulmonary viral titers compared to those fed the control diet (10^6.7 vs 10^4.8, p < .05) and were able to maintain their body weight after infection (1.8 ± 0.9 g weight loss/5 days postinfection in vitamin E group vs 6.8 ± 1.4 g weight loss/5 days postinfection in control group, p < .05). Other antioxidants did not have a significant effect on viral titer or weight loss. There was a significant inverse correlation of weight loss with food intake (r = -.96, p < .01), indicating that the observed weight changes were mainly due to decreased food intake. Pulmonary interleukin (IL)-6, IL-1, and tumor necrosis factor (TNF)-α levels increased significantly postinfection. The vitamin E group had lower lung IL-6 and TNF-α levels following infection compared to the control group. In addition, there was a significant positive correlation between weight loss and lung IL-6 (r = .77, p < .01) and TNF-α (r = .68, p < .01) levels. Because IL-6 and TNF-α have been shown to contribute to the anorexic effect of infectious agents, the prevention of weight loss by vitamin E might be due to its reduced production of IL-6 and TNF-α following infection. Thus, among the antioxidants tested, only vitamin E was effective in reducing pulmonary viral titers and preventing an influenza-mediated decrease in food intake and weight loss. Other dietary antioxidant supplementations that reduced one or more measures of oxidative stress (4-hydroxynonenal, malondialdehyde, and hydrogen peroxide) did not have an effect on viral titer, which suggests that, in addition to its antioxidant activity, other mechanisms might be involved in vitamin E’s beneficial effect on lowering viral titer and preventing weight loss.

The incidence of infectious diseases increases with age and is associated with higher morbidity and mortality. As infectious diseases may require hospitalization, they contribute greatly to the medical costs associated with the care of elderly people. Respiratory infections are particularly common (1), and influenza viruses are the most challenging when development of more effective preventive strategies is considered: they change antigenic character at irregular intervals, infect individuals of all ages, produce yearly epidemics associated with excess morbidity and mortality, and predispose the host to bacterial superinfections (2).

Influenza virus predominantly damages epithelial cells of the lung and airways. Although the molecular mechanisms of influenza virus pathogenesis are still largely obscure, possible mechanisms include apoptosis and generation of reactive oxygen species (ROS) (3–5). Neutrophils and macrophages produce superoxide (O^−2) and hydrogen peroxide (H_2O_2), which normally are involved in the killing of ingested or invading microbes. However, these ROS are toxic to a wide variety of eukaryotic cells and could contribute to the tissue damage seen in lungs after influenza virus infection (3,6).

Generation of ROS by alveolar phagocytic cells increases with influenza virus infection (7). Other than phagocyte-generated ROS, influenza virus itself is involved in the direct generation of ROS. Pahl and Baueuerle (8) reported that expression of influenza virus hemagglutinin (HA) activates nuclear transcription factor (NF)-κB DNA binding and transactivation in HeLa and 293 cells. Activation was inhibited in the presence of the antioxidant dithiothreitol, suggesting that HA increases the production of ROS within the cell, which may act as second messengers to activate NF-κB. Other than this direct role in viral pathogenesis, oxidative stress can also have indirect effects on the infectivity of influenza virus by influencing the balance of protease/antiprotease or by influencing the interplay of cytokines such as tumor necrosis factor (TNF), interleukin (IL)-1, or interferons (IFNs) (9).

A wide variety of systems contribute to cellular antioxidant defense, including such enzymes as superoxide dismutase,
catalase, glutathione peroxidase, and small molecules such as vitamin E, vitamin C, and glutathione. Jacoby and Choi (10) showed that viral infection increases the expression of the antioxidant enzymes manganese superoxide dismutase (MnSOD) and indoleamine 2,3-dioxygenase (IDO), which may be an important defense against oxygen radical-mediated damage. Hennet and colleagues (4) showed that influenza infection resulted in a decrease in the total concentrations of the antioxidants glutathione and vitamin E from lung and liver tissues. This loss of antioxidants may be due to increased production of oxidants.

In a previous study (11), we showed that old mice whose diet was supplemented with 500 ppm vitamin E for 6 weeks had significantly lower lung viral titer than old mice fed control levels (30 ppm) of vitamin E following influenza infection. Vitamin E supplementation preserved the antioxidant status in tissues following influenza virus infection, and a highly significant inverse correlation was observed between viral titers and vitamin E levels. Recently, melatonin, in addition to its hormonal function, has been recognized as having antioxidant capacity in vitro (12). Further, supplementing C57BL/6 mice with melatonin was shown to improve immune function and longevity in middle-aged mice (13). Glutathione, another antioxidant, has also been shown to increase cell-mediated immunity in old mice (14). The phenolic compounds of fruits and vegetables have been shown to have antioxidant capacity. Strawberry extract was shown to have the highest in vitro antioxidant capacity among the 12 fruits tested using the automated oxygen radical absorbance capacity (ORAC) assay (15). Therefore, we tested the efficacy of these antioxidants compared to that of vitamin E on the clinical manifestations (pulmonary viral titers and weight loss) of influenza infection. We hypothesized that if the effect of vitamin E was through its antioxidant function, other antioxidants would show a similar effect.

Materials and Methods

Animals and Infection

Pathogen-free male C57BL/6NcrlBR mice (18 months old) bred in our colony were individually housed in filtered cages in an environmentally controlled atmosphere (temperature 23°C; 45% relative humidity) with 15 air changes per hour and a 12/12 hour light-dark cycle. Mice (15 mice/group) were fed ad libitum with one of the following semi-purified diets for 6 months: control diet (30 ppm vitamin E), vitamin E-supplemented diet [control + 470 ppm vitamin E (α-tocopherol acetate, Harlan Teklad, Madison, WI)], glutathione-supplemented diet [control + 0.5% glutathione (Kyowa Hakko Kogyo Co., Tokyo, Japan)], vitamin E + glutathione-supplemented diet [control + 470 ppm vitamin E + 0.5% glutathione], melatonin-supplemented diet [control + 11 ppm melatonin (Sigma Chemical Co., St. Louis, MO)], or strawberry extract-supplemented diet (control + 1% strawberry extract). Strawberry extract was prepared from fresh stock of strawberries as described (15). The levels of antioxidants were chosen based on our previous experiments (11,14) or those reported in the literature (13). Control diet (all ingredients by wt) contained 33.55% corn starch, 33.40% sucrose, 5% cellulose, 3.5% AIN-76A salt mixture, 1% AIN-76A vitamin mixture, 0.3% D-L methionine, 0.25% choline bitartrate (Harlan Teklad), 18% vitamin-free casein (Bioserve, Frenchtown, NJ), and 5% vitamin E-stripped soybean oil (Bioserve). All conditions and handling of the animals were approved by the Animal Care and Use Committee at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University and followed NIH Guidelines for the Care and Use of Laboratory Animals.

After 6-month dietary supplementation, 10 mice from each diet group were euthanized via CO2 asphyxiation and used as preinfection or day 0 group for different measurements. In addition, 5 mice from each diet group were infected with influenza A/Port Chalmers/1/73 (H3N2) virus according to the method of Bender and colleagues (16). Mice were anesthetized with an intraperitoneal injection of 0.4 mg pentobarbital and infected intranasally with 20 μL of H3N2 virus (TCID50 106.5/10 μL). Mice were euthanized via CO2 asphyxiation 5 days after infection. Day 5 postinfection was chosen because a significant inverse correlation between liver vitamin E level and viral titer previously occurred on that day (11). Weight loss due to influenza infection was calculated using the weight measured on the day before viral challenge and the weight measured before animals were euthanized. Food intake was calculated by subtracting the weight of leftover food in each cage (one mouse per cage) from the food provided. Specialized food containers that restrict food spillage (Lab Products, Maywood, NJ) were used to reduce food loss due to digging.

Tissue Preparation

Blood was drawn by cardiac puncture and the plasma separated and stored at −70°C for later analysis of cytokine levels. The lungs were processed as previously described (17), cut into small pieces, and incubated at 37°C for 1 hour in 6 mL of Iscove’s medium with 10% fetal calf serum (FCS) and 40 U/mL of collagenase (Sigma). Lung pieces were ground and washed through a 60-mesh stainless steel screen. After centrifugation at 1000 rpm for 5 minutes, supernatant was removed and frozen for virus quantification and IL-6, IL-1β, and TNF-α measurements. Lung cells were resuspended and red blood cells were lysed using lysing buffer (Sigma). Lung cells were then counted and cultured for H2O2 production.

Measurements

Determination of lung virus titer.—Lung virus titer was determined by inoculation of lung supernatant into Madin Darby canine kidney cells in round-bottom 96-well plates (16). The TCID50 was calculated using the method of Reed and Muench (18).

Determination of hydrogen peroxide (H2O2) production.—H2O2 was measured after lung cells (106 cells) were stimulated with 1 mg/mL (final concentration) of zymosan (Sigma) for 1 hour at 37°C. Production of H2O2 was measured by H2O2-dependent oxidation of homovanillic acid (3-methoxy-4-hydroxyphenylacetic acid, HVA) to a highly fluorescent dimer (2,2′-dihydroxy-3,3′-dimethoxydiphenyl-5,5′-dicarboxylic acid), which is mediated by horseradish peroxidase (19). A linear relationship between fluorescence (λex =
312 nm and $\lambda_{em} = 420$ nm) and amount of $H_2O_2$ was found in the range of 0.1–10 nmol.

**Determination of liver lipid peroxidation.**—Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) were measured as markers of lipid peroxidation in the liver using LPO-586 kit (R&D Systems, Minneapolis, MN).

**Vitamin E, glutathione, and melatonin assessment.**—Status of vitamin E in control, vitamin E, and vitamin E + glutathione (GSH) groups was assessed by measuring $\alpha$-tocopherol content of plasma by high-performance liquid chromatography using electrochemical detector (20). Status of glutathione was assessed by measuring GSH level of liver by using glutathione S-transferase method (21). Because there was a limitation in availability of lung samples, we measured liver content of GSH and plasma content of $\alpha$-tocopherol.

Previous reports have shown that liver content of GSH correlates with GSH level in lung following influenza infection (4). Furthermore, using similar dietary treatments, we have shown that there is a good correlation between plasma and lung levels of $\alpha$-tocopherol (22). Melatonin-level in plasma of control and melatonin-supplemented groups was measured by radioimmunoassay using a commercially available kit (Elias, Osceola, WI).

**IL-6, IL-1β, and TNF-α determination.**—IL-6, IL-1β, and TNF-α were measured by ELISA according to the manufacturer’s instruction using rat anti-mouse IL-6 or TNF-α monoclonal antibodies (PharMingen, San Diego, CA) or rat anti-mouse IL-1β polyclonal antibody (Endogen, Cambridge, MA) and biotinylated rat anti-mouse IL-6, TNF-α, or IL-1β antibodies.

**Statistical Analysis**

Data were analyzed by analysis of variance (ANOVA) for overall effects of diet followed by Fisher’s least significant difference post hoc test for individual comparisons using the SYSTAT 1992 statistical package (SYSTAT, Evanston, IL). Student’s paired t test was used to test for differences in body weights before and after infection. Kruskal-Wallis nonparametric test was used for data that were not normally distributed. Pearson correlation was used to determine association. Data are reported as mean $\pm$ SEM. Significance was set at $p < .05$. Sample size was calculated to achieve 30% difference in influenza titer with $\alpha = .05$ and 80% power using mean and standard deviation from our previous study (11).

**RESULTS**

**Lung Viral Titer and Prevented Weight Loss Following Influenza Virus Infection**

As shown in Table 1, animals fed vitamin E or vitamin E + GSH had significantly higher plasma vitamin E levels compared to those fed the control diet. Animals fed the melatonin diet showed a significant increase in plasma melatonin levels compared to those fed the control diet. Animals fed glutathione-supplemented diet did not have significantly different liver glutathione levels compared to those fed the control diet. This may be due to the fact that the glutathione level was measured from uninfected animals. Most normal cells have a large excess of glutathione. However, decreased tissue concentration of glutathione has been reported in several disease states including Wilson’s disease, HIV infection, and influenza virus infection (4,23). Increased splenocyte-SH content occurred in mice fed 1% GSH only after 68 hours of stimulation with ConA, but no effect of supplementation was seen in the absence of the mitogen (14). Alternatively, the 1% GSH level added to the diet might not have been effective in changing the tightly regulated GSH level in presence of adequate protein levels (24,25). Animals fed vitamin E + GSH supplemented diet, however, did tend to have higher liver GSH levels compared to those fed the control diet ($p = .09$). This might be due to vitamin E’s protective effect against the oxidation of glutathione.

There was a significant effect of diets on lung viral titer (ANOVA, $p = .026$). Lung influenza viral titers were significantly lower ($p = .017$ in the vitamin E-supplemented group compared to the control group (Figure 1). There was no significant difference between viral titers in animals fed the other antioxidant-supplemented diets and those fed the control diet.

There was no significant difference in baseline weight between different dietary groups (Table 2). Weight loss after influenza virus infection in the vitamin E-supplemented group (1.84 + 0.91 g/5 days postinfection) was significantly less ($p = .016$) than that of the group fed the control diet (6.78 + 1.39 g/5 days postinfection). There was no significant difference in weight loss between mice fed the control diet and that of mice fed the other diets. Mice fed the control diet, GSH-supplemented diet, and melatonin-supplemented diet showed significant weight loss following influenza infection ($p = .008$, .008, and .004, respectively) compared to their weight before infection. Vitamin E + GSH-, and strawberry extract-supplemented groups tended to lose weight after infection ($p = .054$ and .065, respectively), whereas mice in the vitamin E group did not exhibit a significant weight loss compared to their weight before infection ($p = .114$). The weight loss appears to be mainly due to decreased food intake, as there was a highly significant inverse correlation ($r = -0.96$, $p < .001$; Figure 2) between weight loss and food intake. Furthermore, the vitamin E-supplemented group had significa-
cantly higher food intake during 5 days postinfection compared to other groups \((p < .05); \text{Table 2}\).

**Malondialdehyde (MDA) and 4-Hydroxynonenal (4-HNE) in Liver**

There was a significant effect of antioxidant supplementation on the levels of lipid peroxidation markers in the liver \((p = .001 \text{ for MDA, } p = .004 \text{ for 4-HNE})\). Mice fed vitamin E- or vitamin E + GSH-supplemented diet had significantly lower levels of MDA and 4-HNE levels in the liver compared to mice fed the control diet. Mice fed the strawberry extract-supplemented diet had significantly lower 4-HNE but not MDA levels than mice fed the control diet (Table 3). GSH or melatonin supplementation did not have significant effects on liver 4-HNE and MDA levels.

**Hydrogen Peroxide Production**

ANOVA results indicate that \(\text{H}_2\text{O}_2\) production by lung cells significantly increased with influenza infection \((2.86 \pm 0.22 \text{ nmoles/mL in Day 0 vs } 3.79 \pm 0.12 \text{ nmoles/mL in Day 5, } p = .001)\). There was no significant overall effect of dietary antioxidant supplementation on \(\text{H}_2\text{O}_2\) production before or following infection. However, \(\text{H}_2\text{O}_2\) production on Day 0 tended to be lower in vitamin E \((2.45 \pm 0.5 \text{ nmoles/mL, } n = 7, p = .076)\) and melatonin \((2.32 \pm 0.45 \text{ nmoles/mL, } n = 5, p = .092)\) groups compared to the control group \((3.63 \pm 0.35 \text{ nmoles/mL, } n = 7)\). Levels of \(\text{H}_2\text{O}_2\) production by GSH \((2.71 \pm 0.35 \text{ nmoles/mL}),\) vitamin E+GSH \((2.94 \pm 1.02 \text{ nmoles/mL}),\) and strawberry \((2.84 \pm 0.74 \text{ nmoles/mL})\) groups on Day 0 were not significantly different from the control group.

**Plasma IL-6 Level**

Plasma IL-6 level increased significantly 5 days after influenza infection in all diet groups \((p < .005); \text{Figure 3}\). There was, however, no significant effect of dietary antioxidant supplementation on plasma IL-6 levels before or after influenza infection. Furthermore, there was no significant correlation between plasma IL-6 levels and weight loss or food intake.

### Table 2. Effect of Dietary Antioxidant Supplementation on Weight Loss and Food Intake Following Influenza Virus Infection

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>Wt at 18 mo</th>
<th>Wt at 24 mo &amp; Before Infection</th>
<th>Wt at 24 mo &amp; After Infection</th>
<th>Wt loss/5 Days &amp; Postinfection</th>
<th>Food Intake/5 Days &amp; Postinfection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.44 ± 2.68</td>
<td>42.30 ± 2.44</td>
<td>35.54 ± 1.75*</td>
<td>6.78 ± 1.39</td>
<td>6.28 ± 3.04</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>39.60 ± 2.95</td>
<td>45.98 ± 4.27</td>
<td>44.14 ± 3.89</td>
<td>1.84 ± 0.91*</td>
<td>16.86 ± 2.60†</td>
</tr>
<tr>
<td>Vitamin E + Glutathione</td>
<td>46.36 ± 4.56</td>
<td>57.40 ± 3.42</td>
<td>52.34 ± 3.28†</td>
<td>5.06 ± 1.88</td>
<td>7.92 ± 1.55</td>
</tr>
<tr>
<td>Glutathione</td>
<td>38.20 ± 1.81</td>
<td>44.96 ± 4.44</td>
<td>39.98 ± 4.65*</td>
<td>4.98 ± 1.00</td>
<td>9.24 ± 3.14</td>
</tr>
<tr>
<td>Melatonin</td>
<td>41.04 ± 5.40</td>
<td>55.40 ± 2.50</td>
<td>49.64 ± 2.52*</td>
<td>5.76 ± 0.99</td>
<td>7.52 ± 2.39</td>
</tr>
<tr>
<td>Strawberry</td>
<td>34.55 ± 1.49</td>
<td>49.63 ± 4.09</td>
<td>44.23 ± 2.46†</td>
<td>5.40 ± 1.89</td>
<td>7.80 ± 4.12</td>
</tr>
</tbody>
</table>

Notes: Values are mean ± SEM; \(n = 5\).

*Significantly different from weight before infection by Student’s paired \(t\) test \((p < .05)\).

† Different from weight before infection by Student’s paired \(t\) test \((p < .1)\).

‡ Significantly different from control group by Fisher’s least significant test \((p < .05)\).
Lung IL-1β, IL-6, and TNF-α Levels

ANOVA results indicate that there was a significant effect of influenza infection on IL-1β levels in lung homogenate (p < .001). There was no significant effect of dietary antioxidant supplementation on lung IL-1β levels before or after influenza infection.

As shown in Figure 4, IL-6 levels in lung homogenate increased significantly with infection (p < .001). Overall, there was no effect of dietary antioxidant supplementation on IL-6 levels and no significant interaction between infection and diet. However, as only the vitamin E group showed lower viral titer, confirming our previous observations (11), and no significant weight loss following infection, IL-6 levels in each group were compared to that of the control group using a post-hoc test. IL-6 levels following infection were significantly lower in the vitamin E group (1519.5 ± 496.2 pg/mL) compared to the control group (3372.5 ± 419.2 pg/mL, p = .009). Also, a significant positive correlation (r = .77, p < .001) was observed between IL-6 levels and weight loss (Figure 5).

There was a significant effect of infection on TNF-α levels (ANOVA, p < .005; Figure 6). There was no main effect of dietary antioxidant supplementation and no significant interaction between infection and diet. However, because TNF-α has been shown to have significant effects on appetite and body weight, and only the vitamin E group showed no significant weight loss and significantly higher food intake, TNF-α levels in each group were individually compared to

Table 3. Effect of Dietary Antioxidant Supplementation on Liver 4-Hydroxynonenal (4-HNE) and Malondialdehyde (MDA) Levels

<table>
<thead>
<tr>
<th>Diet Group</th>
<th>4-HNE (nM/g Liver)</th>
<th>MDA (nM/g Liver)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.35 ± 0.28</td>
<td>1.68 ± 0.21</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>1.33 ± 0.11*</td>
<td>1.00 ± 0.12*</td>
</tr>
<tr>
<td>Vitamin E + Glutathione</td>
<td>1.44 ± 0.13*</td>
<td>0.89 ± 0.14*</td>
</tr>
<tr>
<td>Glutathione</td>
<td>1.89 ± 0.16</td>
<td>1.39 ± 0.14</td>
</tr>
<tr>
<td>Melatonin</td>
<td>2.18 ± 0.30</td>
<td>1.53 ± 0.32</td>
</tr>
<tr>
<td>Strawberry</td>
<td>1.57 ± 0.12*</td>
<td>1.14 ± 0.11</td>
</tr>
</tbody>
</table>

Notes: Values are mean ± SEM; n = 16–20.
*Significantly different from control group by Kruskal-Wallis nonparametric test corrected for multiple comparison by Bonferroni adjustment (p < .05).
that of the control group using a follow-up test. TNF-α levels following infection were significantly lower in the vitamin E group (1.66 ± 0.12 U/mL, *p* = .033) and melatonin group (1.62 ± 0.34 U/mL, *p* = .030) compared to the control group (3.65 ± 0.46 U/mL). A significant positive correlation (*r* = .68, *p* < .001) between TNF-α levels and weight loss was observed (Figure 7).

**DISCUSSION**

Influenza virus is a significant human pathogen. The clinical expression of infection with influenza virus is variable and greatly influenced by the age, physiological state, and immunological experience of the host. Morbidity and mortality during influenza epidemics are particularly high among elderly people (26). The increased susceptibility of elders to influenza virus infection can be attributed to several factors, including age-associated decline of cell-mediated immune and humoral immune responses and increased oxidative stress with aging. Oxidative stress has been shown to play a role in both pathogenesis and infectivity of influenza virus (4,9,27).

Vitamin E, a potent biological antioxidant, has been shown to enhance cell-mediated and humoral immune responses in aged mice and humans (28,29). We previously demonstrated that vitamin E supplementation in old mice resulted in a significant reduction of lung influenza virus titer after influenza infection (11). In the current study, we tested the effect of long-term supplementation with vitamin E and other dietary and nondietary antioxidants on the course of influenza infection. Of the antioxidants tested, only vitamin E supplementation was effective in decreasing lung viral titer and preventing the weight loss and decreased food intake typically observed with influenza virus infection. GSH, which has been shown to play an important role in the detoxification of ROS and xenobiotics and in regulating immune function (23,30), had no effect on lung viral titer. Melatonin, which acts as an intracellular scavenger of hydroxyl and peroxyl free radicals (12,31) and enhances immune response (32), tended to reduce H₂O₂ production by lung cells, but did not have a significant effect on liver MDA and 4-HNE levels or on lung influenza viral titer. This difference might be due to the differences in the dose (11 mg/kg diet vs 5–10 mg/kg b.w.) or administration route of melatonin [oral vs i.p. injection; (31,32)]. Strawberry extract has been shown to have high antioxidant activity in vitro, as measured by the ORAC assay (15). Animals fed strawberry extract-supplemented diet had significantly lower levels of liver 4-HNE compared to those fed the control diet, yet supplementation with strawberry extract did not affect lung influenza viral titer.

This lack of effect by other antioxidants on lung viral titer might represent the difference in biological availability of these compounds compared to vitamin E. On the other hand, this lack of an effect suggests that factors other than antioxidant activity might have contributed to the protective effect of vitamin E against influenza infection. This is supported by the observation that viral titers of the animals fed vitamin E + GSH were similar to those of the control, whereas animals fed vitamin E alone had significantly lower viral titer than those fed the control diet despite a similar effect of the two treatments on 4-HNE and MDA levels in the liver. Although hepatic GSH levels tended to be slightly higher in animals fed vitamin E + GSH than in animals fed GSH alone, this higher GSH status did not render any detectable beneficial effects against infection. Rather, GSH abolished vitamin E’s beneficial effect. This indicates that there is an interaction between GSH and vitamin E.

H₂O₂ production in lung increased following influenza infection in this study. Buffinton and colleagues (27) also
reported increased H$_2$O$_2$ in the lungs of mice suffering from influenza. Increased production of oxidants can decrease viral clearance by suppressing the immune response. In addition, increased oxidative stress can enhance viral titer by inactivating the antiprotease that affects the activation of viruses by proteolytic cleavage (4,9). In this study, there was no difference in H$_2$O$_2$ production following influenza infection (Day 5) between different dietary groups. On Day 0, vitamin E and melatonin groups tended to have lower H$_2$O$_2$ production compared to the control group. However, only the vitamin E group had significantly lower viral titer. Taken together, these results indicate that vitamin E’s effect on lowering viral titer is not solely due to its antioxidant property. Viral infection results in acute phase responses including lethargy, fever, and anorexia (33). In our study, significant weight loss due to anorexia was observed in all experimental groups except the vitamin E-supplemented group over the 5 days postinfection. The vitamin E-supplemented group consumed about twice as much food and showed about one third of the weight loss of the other groups. The mechanisms by which the host triggers the acute phase responses are not known. Cytokines such as IL-1, IL-6, TNF-α, and IFN, produced upon viral infection, are capable of causing both fever and anorexia (33,34). Serum IL-6 levels were shown to be higher in aged than in young mice and to increase following influenza infection (34,35). Thivierge and Rola-Pleszczynski (36) showed that prostaglandin (PG)E$_2$ can increase the production of IL-6 by macrophages, and vitamin E has been shown to decrease PGE$_2$ and IL-6 production (29,37). There was no overall effect of dietary antioxidant supplementation on lung IL-6 levels, but mice in the vitamin E group showed significantly lower lung IL-6 levels than those in the control group on Day 5, postinfection. In addition, a significant positive correlation was observed between lung IL-6 levels and weight loss following influenza infection. However, we did not observe a significant effect of antioxidant supplementation on plasma IL-6 levels in this study. In addition, no significant correlation between weight loss and plasma IL-6 levels was observed. Thus, the beneficial effect of vitamin E on weight loss could be explained by the difference in lung IL-6 levels following infection but not by a change in plasma IL-6 levels.

Vitamin E, added in vitro, has been shown to inhibit endotoxin-induced production of TNF-α by alveolar macrophages and Kupffer cells (38,39). Dietary supplementation of vitamin E in mice infected with LP-BM5 retrovirus has been shown to decrease the virus-induced elevation of TNF-α production by lipopolysaccharide (LPS)-stimulated splenocytes (40). In our study, lung TNF-α levels increased with infection. The vitamin E group had lower levels of pulmonary TNF-α than the control group after infection although there was no overall effect of dietary antioxidant supplementation on lung TNF-α levels. In addition, a significant positive correlation was observed between lung TNF-α levels and weight loss. TNF-α was not detectable from most of the plasma samples (18/26) in this study (data not shown). Thus, the effect of vitamin E on weight loss might have been mediated through a reduction in TNF-α and IL-6 production. There was a significant positive correlation between viral titer and lung IL-6 levels ($r = .54$, $p = .004$) and a less significant positive correlation between viral titer and lung TNF-α ($r = .33$, $p = .09$). TNF-α has a dual role in influenza infection. While it has been shown to have an antiviral property (41), its higher production following infection contributes to pathogenesis of influenza including loss of appetite and weight loss (33,42). Thus, while the reduction of TNF-α by vitamin E can contribute to its effect on weight loss, it cannot explain vitamin E-induced reduction of viral titer. Similarly, while reduction of IL-6 by vitamin E supplementation contributes to maintenance of weight, it cannot explain its effect on viral titer, as IL-6 is not known to have direct antiviral effect.

Another possible mechanism of vitamin E’s effect on food intake could be suppression of cyclooxygenase products, particularly PGE$_2$. Macrophages and spleens from old mice have significantly higher production of cyclooxygenase products (43,44). We have previously shown that vitamin E can inhibit cyclooxygenase activity and decrease PGE$_2$ production using similar dietary treatments (29,45). Shimomura and colleagues (46) showed that suppressed food intake by peripheral administration of IL-1 was partially restored to control levels by ibuprofen, a selective cyclooxygenase inhibitor. Future studies are planned to determine the contribution of IL-6, TNF-α, and PGE$_2$ in vitamin E-induced prevention of weight loss following influenza infection.

In conclusion, we have demonstrated that long-term vitamin E supplementation is effective in lowering viral titer and preventing decreased food intake and weight loss following influenza infection in aged mice. This beneficial effect was not observed with the other antioxidants tested. Vitamin E’s effect on prevention of weight loss following influenza infection appears to be due to reduction in lung IL-6 and TNF-α levels. While the mechanism of vitamin E’s protective effect on viral titer needs to be delineated, these results suggest that mechanisms other than antioxidant protection are likely to be involved.

Acknowledgments

This research was based upon work supported by the U.S. Department of Agriculture, under Agreement 58-1950-9-001. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture.

The authors thank Dr. Alison A. Beharka and Robert Cottee for technical assistance and Joanne Meegan for preparation of the manuscript.

Address correspondence to Dr. Simin Nikbin Meydani, Nutritional Immunology Laboratory, Jean Mayer Human Nutrition Research Center on Aging at Tufts University, 711 Washington Street, Boston, MA 02111. E-mail: smeydani@hnrc.tufts.edu

References


