Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies1–3

Peter Møller and Steffen Loft

ABSTRACT Many epidemiologic studies have addressed the possible preventive effects of antioxidants in disease causation and progression. With the use of molecular techniques, it is feasible to investigate specific properties of antioxidants in intervention studies. The most widely used techniques to investigate oxidative DNA damage in white blood cells are the measurement of 7-hydroxy-8-oxo-2′-deoxyguanosine and the comet assay. The types of antioxidant intervention studies include those involving single or multiple supplemetations of vitamin C, vitamin E, or carotenoids and those involving various natural food products (eg, carrot juice). In short-term intervention studies (usually weeks or a few months), results have been mixed. Single-dosing studies found that decreased oxidative DNA damage lasted only hours after antioxidant supplementation, suggesting that the preventive effect is relatively short. In addition, many of the positive studies were not placebo-controlled, thus leaving a possibility of false-positive results caused by period effects, eg, seasonal variation, changes in the lifestyles of the subjects, or variation in measurements over time. Because participation in an antioxidant intervention study may cause changes in dietary habits and because seasonal changes may have profound effects, it is recommended that future studies have a placebo-controlled, parallel design rather than a crossover design.

KEY WORDS Antioxidants, comet assay, humans, intervention studies, 7-hydroxy-8-oxo-2′-deoxyguanosine, oxidative DNA damage, vitamin C, vitamin E, carotenoids

INTRODUCTION The cells of the human body are continuously attacked by reactive oxygen species (ROS), which arise as natural byproducts of normal cellular energy production or are generated in large amounts by exhaustive exercise or by chemical agents in the environment (1). In addition, cancer and chronic inflammatory diseases generate ROS as part of the pathophysiologic mechanism (2). Cells have evolved a complex network of defense barriers to counteract the generation of ROS and protect against the oxidation of macromolecules by scavenging ROS. The dietary intake of antioxidants is thought to play a major role in this network. Some compounds that have antioxidant properties, such as vitamin C, vitamin E, carotenoids, and flavonoids, have been identified in large quantities in some natural food products (3). However, these antioxidants are few in comparison with the many other antioxidants and other bioactive substances in natural products.

The antioxidant capacity of fruit and vegetables plays an important role in their protective effect (4, 5). From epidemiologic studies it has been concluded that lifestyles characterized by a high consumption of fruit and vegetables are associated with lower incidences of cancers of the lung, stomach, breast, colorectum, oropharynx, and liver (6). However, the large antioxidant intervention studies did not show a decreased cancer risk associated with antioxidant consumption (7–9).

An alternative approach to investigate antioxidant effects is to study the behavior of oxidative stress biomarkers in controlled supplementation studies. Using DNA as the biological target molecule, 2 methods appear to be the tests of choice: measurement of 7-hydroxy-8-oxo-2′-deoxyguanosine (8-oxodG) and the comet assay. From a mechanistic perspective, dietary antioxidants are expected to inhibit the formation of oxidative DNA damage. When ROS are formed in vitro or by oxidative stress in vivo, several types of oxidative DNA lesions are formed, including small base lesions and exocyclic adducts (10). 8-OxodG is one of the most easily formed oxidative DNA lesions. It can be detected in both urine and tissues after oxidative stress (11). 8-OxodG can be measured with the use of several chromatographic techniques, including HPLC with electrochemical detection and tandem mass spectrometry (MSMS), gas chromatography–mass spectrometry (GC-MS), and thin-layer chromatography with 32P postlabeling, and with antibody-based immunoassays (12). There is large variation between the different assays in the measured concentrations of 8-oxodG, partly because of artificially generated 8-oxodG in the assays (13). In particular, GC-MS–based methods produce high values because of artifacts. Recently, however, a joint effort of laboratories mainly in Europe narrowed down the range of measured values of 8-oxodG obtained by the various methods (13). 8-OxodG was probably chosen as the prime oxidative DNA-damage biomarker because it can be sensitively measured by HPLC with electrochemical detection and because it appears to be more closely linked with mutagenesis than are, for example, oxidative adenine and thymine lesions (11). For other types of oxidative DNA damage, standardization of laboratory procedures and agreement on basal values are still warranted.

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The comet assay detects DNA strand breaks (SBs). An enzyme-modified version of the comet assay was developed to detect oxidatively altered nucleotide bases by including a DNA digestion step using DNA glycosylase enzymes (14). Oxidized purines, including 8-oxodG, can be detected by formamidopyrimidine-DNA glycosylase (EC 3.2.2.23), and oxidized pyrimidines by endonuclease III (ENDOIII; EC 4.2.99.18). In addition, in vitro exposure of cells to DNA-breaking agents, such as hydrogen peroxide or ionizing radiation, has been used as a semiquantitative measurement of the donor’s antioxidative status. This modification of the comet assay is based on the notion that the intracellular content of antioxidants will inhibit DNA breakage.

Here, we review antioxidant supplementation studies in which oxidative DNA damage was detected in leukocytes, lymphocytes, or mononuclear blood cells. We chose to limit our review to only those studies in which damage was assessed in blood cells because most studies assessed damage in that cell population. We also limited the survey to only those studies in which damage was detected either by the comet assay (or related assays) or by measurement of 8-oxodG. Data from assays that require the growth of cells, eg, assays that detect micronuclei or chromosomal aberrations, are not included in the present review. Moreover, other types of lesions were included in very few studies, and potential methodologic problems in those studies (eg, possible artifacts for some lesions) limit the interpretation of their results. An article featuring an extensive review of urinary 8-oxodG excretions was recently published (12). In addition, reviews by Halliwell (15) and Collins (16) summarizing the effects of antioxidants on biomarkers will inhibit DNA breakage.

### SINGLE-DOsing ANTIOXIDANT INTERVENTION STUDIES

A summary of the results of single-dosing antioxidant supplementation studies is provided in Table 1. Vitamin C supplementation decreased the number of SBs as well as the sensitivity to hydrogen peroxide and ionizing radiation (18, 19). The effect of vitamin C appeared to peak early, within 2–8 h after consumption, and smokers benefited more from dietary vitamin C supplementation than did nonsmokers (19). Consumption of vitamin E or β-carotene decreased the number of SBs and hydrogen peroxide sensitivity 18–24 h after ingestion (19). Decreased 8-oxodG concentrations were observed 6 h after consumption of tomatoes (8 g/kg body wt) (21). The later onset of the carotenoid-related effect may have been because of a slower uptake of these antioxidants than of the water-soluble vitamin C.

Consumption of 200 g lightly fried onions/d as a source of flavonoids decreased the number of SBs and hydrogen peroxide sensitivity, whereas the number of ENDOIII sites was apparently unchanged (17). When uncooked cherry tomatoes were added to the onion diet, there were fewer ENDOIII sites but there was no decrease in the number of SBs or in hydrogen peroxide sensitivity. In any case, the effects on the biomarkers were apparently transient because they were observed 4–8 h after consumption but not at 24 h (17). A study of kiwi fruit juice, in which subjects consumed a single dose of 500 mL, showed decreased sensitivity to hydrogen peroxide, with a maximum after 8 h, but no effects on the number of SBs or ENDOIII sites (20).

### MULTIPLE-DOsing, SHORT-TERM ANTIOXIDANT INTERVENTION STUDIES

Most antioxidant supplementation studies included intervention periods of weeks or months. For the sake of clarity, the studies are divided into single and multiple antioxidant studies and studies using natural food products. To date, 26 studies using either the comet assay or measurement of 8-oxodG have been published. Details of the studies are provided in Tables 2 (comet assay) and 3 (8-oxodG).

#### Single antioxidant trials

Studies of vitamin C supplementation provide conflicting results. Supplementation with vitamin C (500 mg/d) for 3 wk decreased 8-oxodG concentrations as assessed by GC-MS after 3 and 6 wk in nonsmokers (36, 48). However, instead of a random

### TABLE 1

<table>
<thead>
<tr>
<th>Study design and type of antioxidant</th>
<th>Assay</th>
<th>n2</th>
<th>Age1</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sequential study of 200 g onions/d, with and without 100 g cherry tomatoes/d, and a 1-wk washout in between</td>
<td>Comet 6 (all ns) 20–44</td>
<td>Decreased hydrogen peroxide sensitivity (4 and 8 h) and fewer SBs (8 h) but no effect on the number of ENDOIII sites (0–24 h) in the onion group; fewer ENDOIII sites (4 and 8 h) but no effect on the number of SBs (0–24 h) or on hydrogen peroxide sensitivity (0–24 h) in the onion–cherry tomato group</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast and vitamin C (35 mg/kg body wt) after an overnight fast</td>
<td>Comet 6 (1 s) 32–58</td>
<td>Decreased sensitivity to ionizing radiation and fewer SBs (peak by 4 h) for vitamin C</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sequential vitamin C (1 g), vitamin E (1 g), and β-carotene (45 mg) with a 1-wk washout in between</td>
<td>Comet 12 (6 s) 20–32</td>
<td>Fewer SBs (2–4 h; normal by 6 h) and decreased hydrogen peroxide sensitivity (2 h) for vitamin C; fewer SBs (18–24 h) and decreased hydrogen peroxide sensitivity (24 h) for vitamin E and β-carotene</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single 500-mL dose of kiwi fruit juice Tomatoes (8 g/kg body wt) after a 12-h overnight fast and a 2-d washout</td>
<td>Comet 6 (all ns) 24–55</td>
<td>Decreased sensitivity to hydrogen peroxide</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>8-oxodG 5 women 27 ± 7</td>
<td>Decreased 8-oxodG concentrations 6 h after ingestion (P = 0.07, paired t test)</td>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Number of smokers (s) or nonsmokers (ns) in parentheses.
2 Range or ± SD.
sequence, the placebo treatment was carried out before the vitamin C supplementation period. In a placebo-controlled crossover study, subjects who received either 60 mg or 6 g vitamin C/d for 2 wk had increased plasma concentrations of vitamin C; yet, there was no effect on the number of SBs or on hydrogen peroxide sensitivity (27). In an exceptionally well-controlled trial, subjects ate identical meals that initially included 250 mg vitamin C/d, and thereafter the dose of vitamin C was gradually reduced to 5 mg/d and maintained for 92 d; there was no effect on 8-oxodG concentrations, decreased hydrogen peroxide sensitivity, no effect on the number of SBs or on hydrogen peroxide sensitivity, despite increased vitamin E concentrations in plasma (29). The effect of vitamin E was studied in combination with a diet either high or low (15% or 5% as food energy) in polyunsaturated fatty acids (PUFAs) among nonsmoking men (30). The results of this study indicated that the PUF content had no effect on hydrogen peroxide sensitivity or on the number of SBs or ENDOIII sites in subjects supplemented with 80 mg vitamin E/d for 4 wk. However, in subjects who were supplemented with only 5–7 mg vitamin E/d, hydrogen peroxide sensitivity and the number of ENDOIII sites decreased when eating a 5% PUFA diet, whereas the same endpoints increased after consumption of the 15% PUFA diet (30). In a similar study, Chen et al (38) found no differential effect on 8-oxodG concentrations between groups receiving a high–vitamin E–low-PUFA diet or a low–vitamin E–high-PUFA diet. However, 8-oxodG concentrations decreased gradually in both groups throughout the total study period of 31 d.

Table 2: Multiple dosing of dietary antioxidants with assessment of effects by the comet (or related) assay.

<table>
<thead>
<tr>
<th>Study design and type of antioxidant</th>
<th>n(^2)</th>
<th>Age(^1)</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soya milk, rice milk, or cow milk (1 L/d) for 4 wk and a 2-wk washout in between</td>
<td>10 men (all ns)</td>
<td>20–50</td>
<td>Fewer ENDOIII sites after 4 wk (soya milk); no effect on the number of SBs or on hydrogen peroxide sensitivity</td>
<td>22</td>
</tr>
<tr>
<td>Tomato purée (25 g/d) for 2 wk and a 1-wk washout</td>
<td>11 women (all ns)</td>
<td>25 ± 2</td>
<td>Decreased hydrogen peroxide sensitivity; no effect on the number of SBs</td>
<td>23</td>
</tr>
<tr>
<td>Vitamin C (100 mg/d), vitamin E (280 mg/d), and β-carotene (25 mg/d) for 20 wk</td>
<td>100 men (50 s)</td>
<td>50–59</td>
<td>Fewer ENDOIII sites and decreased hydrogen peroxide sensitivity after 20 wk; no effect on the number of SBs</td>
<td>24</td>
</tr>
<tr>
<td>Sequential tomato juice, carrot juice, and spinach for 2 wk each</td>
<td>23 men (all ns)</td>
<td>27–40</td>
<td>Fewer SBs; fewer ENDOIII sites (carrot juice); no effect on hydrogen peroxide sensitivity</td>
<td>25</td>
</tr>
<tr>
<td>Sequential crossover study with vitamin C (350 mg/d), vitamin E (250 mg/d), β-carotene (60 mg/d), selenium (80 μg as selenite/d), and vitamin C (350 mg/d) and α-tocopherol (250 mg/d) for 4 wk and a 4-wk washout in between supplements</td>
<td>21 men (9 s)</td>
<td>26–59</td>
<td>No effect on the number of SBs or on X-ray sensitivity</td>
<td>26</td>
</tr>
<tr>
<td>Vitamin C (60 mg or 6 g/d) for 2 wk</td>
<td>139 (all ns)</td>
<td>20–68</td>
<td>No effect on the number of SBs or on hydrogen peroxide sensitivity</td>
<td>27</td>
</tr>
<tr>
<td>Vitamin C (1000 mg/d) or vitamin E (800 mg/d) for 42 d</td>
<td>14 (all ns)</td>
<td>26 ± 2</td>
<td>Decreased hydrogen peroxide sensitivity; no effect on the number of SBs</td>
<td>28</td>
</tr>
<tr>
<td>Vitamin E (400 IU/d) for 8 wk</td>
<td>31 (all ns)</td>
<td>40 ± 9</td>
<td>No effect on the number of SBs or on hydrogen peroxide sensitivity</td>
<td>29</td>
</tr>
<tr>
<td>Subjects receiving either a 5% or a 15% PUFA diet supplemented with vitamin E (80 mg/d) or not (5–7 mg/d) for 4 wk</td>
<td>21 men (all ns)</td>
<td>29 ± 1</td>
<td>Low vitamin E group: decreased hydrogen peroxide sensitivity and fewer ENDOIII sites (5% PUFA); increased hydrogen peroxide sensitivity and more ENDOIII sites (15% PUFA); high vitamin E group: no effect on hydrogen peroxide sensitivity or on the number of SBs or ENDOIII sites</td>
<td>30</td>
</tr>
<tr>
<td>Mixture of α- and β-carotene (15 mg/d) for 12 wk</td>
<td>40 (all ns)</td>
<td>25–45</td>
<td>No effect on the number of SBs, ENDOIII sites, or FPG sites</td>
<td>31</td>
</tr>
<tr>
<td>Rye crisp bread (76.5 g/d)</td>
<td>12 women</td>
<td>NR</td>
<td>No effect on the number of SBs, ENDOIII sites, or FPG sites</td>
<td>31</td>
</tr>
<tr>
<td>Sequential supplementation with lutein (15 mg/d), lycopene (15 mg/d), and β-carotene (15 mg/d) for 1 wk and a 3-wk washout</td>
<td>8 (2 s)</td>
<td>24–34</td>
<td>No effect on the number of SBs or on hydrogen peroxide sensitivity at time zero after isolation (possibly fewer SBs in the lycopene supplementation period)</td>
<td>32</td>
</tr>
<tr>
<td>Vegetable-fruit concentrate (500 g/d) for 3 wk and a 2-wk washout</td>
<td>22 men (all s)</td>
<td>18–20</td>
<td>No effect on the number of SBs or ENDOIII sites or on hydrogen peroxide sensitivity</td>
<td>33</td>
</tr>
<tr>
<td>Coenzyme Q(_10) (100 or 300 mg/d) for 1 wk</td>
<td>6 (all ns)</td>
<td>20–50</td>
<td>No effect on the number of SBs, ENDOIII sites, or FPG sites</td>
<td>35</td>
</tr>
</tbody>
</table>

\(^1\)ENDOIII, endonuclease III; SB, strand break; PUFA, polyunsaturated fatty acids; FPG, formamidopyrimidine-DNA glycosylase; NR, not reported.

\(^2\)Range or \(\bar{x} ± SD\).
(38). This probably indicates the effect of subjects entering a controlled dietary intervention study or a seasonal effect.

A study of carotenoid supplementation (lycophene, lutein, or β-carotene) for 1 wk indicated no effect on hydrogen peroxide sensitivity (33). In the 5 subjects who had a 20% increase in plasma lycopene concentration, lycopene supplementation probably decreased the number of SBs (statistics were not included in the manuscript). Lutein and β-carotene supplementation did not alter the number of SBs. Consumption of coenzyme Q10 had no effect on the number of SBs, ENDOIII sites, or formamidopyrimidine-DNA glycosylase activity (33). In the 5 subjects who had a 20% increase in plasma lycopene concentration, lycopene supplementation probably decreased the number of SBs (statistics were not included in the manuscript). Lutein and β-carotene supplementation did not alter the number of SBs. Consumption of coenzyme Q10 had no effect on the number of SBs, ENDOIII sites, or formamidopyrimidine-DNA glycosylase activity (33).

A placebo-controlled crossover study including both smokers and nonsmokers who were given single supplementations of vitamin C (350 mg/d), vitamin E (250 mg/d), β-carotene (60 mg/d), selenium (80 μg/d), or vitamin C (350 mg/d) and vitamin E (250 mg/d) showed no effect on the number of SBs or on X-ray sensitivity after 4 wk of supplementation (26). 8-OxodG concentrations were influenced by β-carotene supplementation: smokers had increased 8-oxodG concentrations after supplementation, whereas nonsmokers had decreased concentrations (26). A small study among smokers (3 subjects per group) found decreased 8-oxodG after vitamin E and red ginseng supplementation, whereas β-carotene and vitamin C had no effect (37). A study in which an enzyme-linked immunosorbent assay was used to detect SBs found decreased hydrogen peroxide sensitivity after supplementation with vitamin C (1000 mg/d) or vitamin E (800 mg/d) for 42 d (28). Consumption of coenzyme Q10 had no effect on the number of SBs, ENDOIII sites, or formamidopyrimidine-DNA glycosylase sites in samples that were analyzed immediately after they were obtained (35).

**Multiple antioxidant trials**

Dietary supplementation with vitamin C (100 mg/d), vitamin E (280 mg/d), and β-carotene (25 mg/d) for 20 wk had no effect on

### Table 3

<table>
<thead>
<tr>
<th>Study design and type of antioxidant</th>
<th>n°</th>
<th>Age</th>
<th>Method</th>
<th>Effect on 8-oxodG</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (500 mg/d) for 6 wk with a 3-wk washout</td>
<td>30 (all ns)</td>
<td>17–49</td>
<td>GC-MS</td>
<td>Decreased after weeks 3 and 6 and normalized after termination</td>
<td>36</td>
</tr>
<tr>
<td>Four groups receiving vitamin C (500 mg/d), vitamin E (200 IU/d), β-carotene (9 mg/d), red ginseng (1.8 g/d), or placebo for 4 wk</td>
<td>15 (all s)</td>
<td>19–31</td>
<td>HPLC</td>
<td>Decreased in the vitamin E (weeks 3 and 4) and red ginseng (week 4) groups</td>
<td>37</td>
</tr>
<tr>
<td>Crossover study with a low vitamin E–high-PUFA diet and a high-vitamin E–low-PUFA diet for 10 d with a 5-d washout period in between supplements</td>
<td>32 (all ns)</td>
<td>31 ± 8</td>
<td>HPLC</td>
<td>No effect related to vitamin supplementation but a decrease in the study period</td>
<td>38</td>
</tr>
<tr>
<td>Mixture of α- and β-carotene (15 mg/d) for 12 wk</td>
<td>40 (all ns)</td>
<td>25–45</td>
<td>HPLC</td>
<td>No effect</td>
<td>31</td>
</tr>
<tr>
<td>Reduced dietary vitamin C content (5 mg/d) for 92 d</td>
<td>8 men (all ns)</td>
<td>25–43</td>
<td>HPLC</td>
<td>No effect</td>
<td>39</td>
</tr>
<tr>
<td>Sequential supplementation with spaghetti sauce (126 g/d), tomato juice (450 mL/d), and oleoresin capsules (2.5 g/d) for 1 wk with a 1-wk washout in between supplements</td>
<td>19 (all ns)</td>
<td>20–40</td>
<td>EC</td>
<td>No effect</td>
<td>40</td>
</tr>
<tr>
<td>Vitamin supplementation (3000 μg β-carotene, 60 mg vitamin C, 30 IU α-tocopherol, 40 mg Zn, 40 μg Se, and 2 mg Cu) for 60 d, with no supplementation 6 wk before the study</td>
<td>19</td>
<td>43 ± 8</td>
<td>HPLC</td>
<td>Decreased after supplementation</td>
<td>41</td>
</tr>
<tr>
<td>Vitamin C (60 or 260 mg/d with 14 g Fe) for 12 wk to subjects stratified by low or high initial plasma vitamin C concentration</td>
<td>38 (all ns)</td>
<td>21–45</td>
<td>GC-MS</td>
<td>Decreased after 6 wk in the high–vitamin C group receiving the low dose of vitamin C</td>
<td>42</td>
</tr>
<tr>
<td>Vitamin (500 mg vitamin C/d, 400 IU α-tocopherol/d, and 12 mg β-carotene/d) or placebo for 6 mo</td>
<td>63 (all s)</td>
<td>42 ± 9</td>
<td>Antibody</td>
<td>No effect between placebo and supplemented groups but a decrease in both groups during the trial</td>
<td>43</td>
</tr>
<tr>
<td>Sequential vitamin C (260 mg/d) with and without iron (14 mg/d) or none (placebo) for 6 wk with an 8-wk washout in between</td>
<td>20 (all ns)</td>
<td>21–45</td>
<td>GC-MS</td>
<td>No effect between placebo and supplemented groups but a decrease in both groups during the trial</td>
<td>44</td>
</tr>
<tr>
<td>Green tea for 1 wk among Chinese and Americans</td>
<td>67 (s + ns)</td>
<td>18–45</td>
<td>HLPC-EC</td>
<td>Decreased in both populations</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>21 men (9 s)</td>
<td>25–59</td>
<td>HPLC</td>
<td>No effect</td>
<td>26</td>
</tr>
<tr>
<td>Vegetable consumption (servings/d: preintervention, 28 women 27–80</td>
<td>28 women</td>
<td>27–80</td>
<td>HPLC</td>
<td>Decreased in subjects with low preintervention plasma α-carotene concentrations</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>5.8; during intervention, 12) for 2 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red wine consumption for 1 mo (240 mL/d) in addition to a high-fat or a Mediterranean diet</td>
<td>42 men</td>
<td>20–27</td>
<td>HPLC</td>
<td>Decreased in both groups</td>
<td>47</td>
</tr>
</tbody>
</table>

1 GC-MS, gas chromatography–mass spectrometry; EC, electrochemical detection.
2 Number of smokers (s) or nonsmokers (ns) in parentheses.
3 Range or x ± SD.
4 Occupationally exposed to tobacco smoke.
the number of SBs (24). However, the number of ENDOIII sites and hydrogen peroxide sensitivity decreased, although at inter-
mediate time points (5 and 10 wk), there was no effect on the num-
ber of ENDOIII sites (24). In a study of subjects who were occu-
pationally exposed to tobacco smoke, 8-oxoG concentrations
decreased after a 60-d supplementation with an over-the-counter
vitamin tablet (3000 mg β-carotene, 60 mg vitamin C, 30 IU
α-tocopherol, 40 mg Zn, 40 mg Se, and 2 mg Cu) (41). Of the
vitamins supplemented, the plasma vitamin C concentration
increased, whereas plasma α-tocopherol and β-carotene concen-
trations remained unaltered (41). A 6-mo vitamin supplementa-
tion trial (500 mg vitamin C/d, 400 IU α-tocopherol/d, and 12 mg
β-carotene/d or placebo) did not show any effect of antioxidant
supplementation; yet, 8-oxoG concentrations decreased in both
groups throughout the trial (more so in the placebo group than in
the supplementation group) (43).

Natural food product trials

Studies of natural food products appear to be different from sin-
gle and multiple antioxidant studies because in the former a wide
range of different food products are investigated, and with the
exception of carotenoid-rich foods, the food products are difficult
to group according to the type of antioxidants that they contain. As
for carotenoid-rich foods, subjects who consumed tomato purée
(25 g/d for 2 wk) in addition to their normal diet had decreased
detoxification of hydrogen peroxide sensitivity, whereas the number of SBs was
unaltered (23). Consumption of other carotenoid-rich food
products (tomato juice, carrot juice, and spinach powder) decreased the number of SBs, whereas ENDOIII sites were
decreased only by drinking carrot juice; hydrogen peroxide sensi-
tivity was unaltered after consumption of any of the
carotenoid-rich foods (25). Unfortunately, neither of these stud-
ies was placebo controlled. A sequential 1-wk supplementation
with spaghetti sauce (126 g/d), tomato juice (450 mL/d), and
decaosyn capsules (2.5 g/d) as sources of lycopene did not pro-
duce a significant effect on 8-oxoG concentrations, although
the authors of the study argued that 8-oxoG concentrations
decreased after the supplementation (40).

Drinking green tea decreased 8-oxoG concentrations in Chi-
nese men who were smokers, and in an American population,
drinking green tea decreased 8-oxoG concentrations more in
smokers than in nonsmokers (45). Drinking red wine (240 mL/d)
decreased 8-oxoG concentrations in 2 groups of men who
received either a high-fat or a Mediterranean diet (47). Consump-
tion of rye crisp bread, a source of phytoestrogenic antioxidants,
for 2 wk had no effect on the number of SBs or ENDOIII sites
(32). Consumption of another source of phytoestrogens, soya
milk, decreased the number of ENDOIII sites after 4 wk of
dietary supplementation, whereas the number of SBs and hydro-
gen peroxide sensitivity remained unchanged (22). A 3-wk
placebo-controlled trial of vegetable and fruit consumption found
that the number of SBs and ENDOIII sites, hydrogen peroxide
sensitivity, malondialdehyde concentrations, and protein oxid-
a tion in male smokers were unchanged, even though plasma con-
centrations of antioxidants and total plasma antioxidant capacity
were increased (34). A stratification of presupplementation plasma α-carotene concentrations into low and high concen-
trations showed that women with low plasma α-carotene concen-
trations had decreased 8-oxoG concentrations after consuming
12 servings of vegetables and fruit/d for 2 wk (compared with 5.8
servings/d at baseline) (46).

Evaluation of study design and power in short-term trials

To allow a critical review of the present short-term studies, we
considered differences in several characteristics as being impor-
tant for a significant outcome, including the type of antioxidant,
the inclusion of smokers, the number of subjects, the ages of the
subjects, the length of the supplementation and washout periods,
the types of detection (for 8-oxoG), and the strength of the study.
Period effects due to seasonal variation or changes in sub-
ject behavior during a trial and unrecognized changes in sampling
or analysis may have profound effects that may severely bias
sequential designs without control groups. Thus, eg, the comet
assay shows covariation with solar flux (49). We defined optimal
studies as randomized, placebo-controlled studies with parallel
designs in which the blood samples from the placebo group were
taken at the same time as those from the supplement group. How-
ever, parallel two-group (antioxidant and placebo) designs may
suffer from baseline differences between the groups that may
affect the outcome measures; ie, it is difficult at the start of a
study to fully normalize 2 groups for antioxidant status, espe-
cially if the groups are small. The second-best choice for a design
is a randomized, placebo-controlled crossover design, although
it is vulnerable to period and carryover effects and a loss of the
statistical power otherwise often inherent in the use of subjects as
their own controls. In that respect, the intrasubject variation in
the comet assay is no less than the intersubject variation (49), and
no statistical power is gained in crossover designs over that of
parallel-group designs. Of the parallel, placebo-controlled stud-
ies, those studies in which samples were taken after the end of the
intervention were considered to have the strongest type of
study design. The strength of the study design was scored from
0 to 3 on the basis of the following criteria: inclusion of a placebo
group or period (1 point), parallel intervention series of placebo
and antioxidant (1 point), and inclusion of samples taken after
the end of the intervention (1 point). This design-strength score
is outlined in Table 4. To evaluate the relation between the strength of the studies and outcome, we performed a statistical
analysis (analysis of variance) with factors, including the bio-
marker method used, the number of subjects, smoking status, the
type of antioxidant (single antioxidant, multiple antioxidants, or
natural products), the duration of the intervention trial and
washout period, and the strength of study design. The statistical
analysis indicated that studies showing protective effects of
antioxidants were more likely to have a low design-strength score
(P < 0.05; n = 26), whereas other factors were nonsignificant. The
mean ± SD strength scores of studies showing protective effects
and those showing no effects were 1.1 ± 1.0 (n = 14) and 1.8 ± 0.6
(n = 12), respectively. A significant reduction in the number of
SBs was only shown in 1 of 14 studies. For ENDOIII sites,
hydrogen peroxide sensitivity, and 8-oxoG, the number of pos-
tive and negative results were evenly distributed: 4 and 4, 4 and
7, and 7 and 6, respectively.

Low power in studies also may explain negative findings. To
investigate this, we calculated how many subjects would be
required to detect a 50% decrease in oxidative DNA damage on
the basis of the mean ± SD values for the control or preinterven-
tion groups (Table 4). This calculation indicated that the positive
and negative studies did not differ (6 of 12 and 6 of 9, respec-
tively) in their power to detect a 50% effect (based on α = 5% and
β = 20% for the difference between 2 groups). In fact, power
to detect smaller effects, eg, differences of 20–30%, would be
desirable.
including 8-oxoadenine, were also observed (42, 44, 48). However, decreases in the level of oxidation of some other DNA bases, GC-MS was used for measurement of 8-oxodG, increases and carotenoids or vitamin E mainly showed no effect at the high (27). Trials investigating oxidative DNA damage with single measurement of 8-oxodG (37, 39, 44) or with the comet assay centrations; however, this trial was sequential, with the placebo Lunec and coworkers (36, 48) indicated decreased 8-oxodG con- cisely because of differences in bioavailability (19).

and carotenoids appear to show protective effects a little later, pos- vitamin C seems to vanish within a few hours, whereas vitamin E idants or natural products (17–21). The effect of a single dose of identified, and all showed some protective effects of single antiox-
more than one-half of a year). Five single-dosing studies were included intervention periods of weeks or a few months (never

DISCUSSION

In this review we distinguish between single-dosing and short-
term studies of protective antioxidant effects in mononuclear blood cells, lymphocytes, or leukocytes. The short-term studies included intervention periods of weeks or a few months (never more than one-half of a year). Five single-dosing studies were identified, and all showed some protective effects of single antioxidants or natural products (17–21). The effect of a single dose of vitamin C seems to vanish within a few hours, whereas vitamin E and carotenoids appear to show protective effects a little later, possibly because of differences in bioavailability (19).

For short-term vitamin C intervention studies, results from Lunec and coworkers (36, 48) indicated decreased 8-oxoG concentra-
tions; however, this trial was sequential, with the placebo period preceding the period with active treatment. In the proper placebo-controlled trials, no effect of vitamin C was observed with measurement of 8-oxoG (37, 39, 44) or with the comet assay (27). Trials investigating oxidative DNA damage with single carotenoids or vitamin E mainly showed no effect at the high doses (26, 29–31, 33, 37, 38). In the studies of vitamin C in which GC-MS was used for measurement of 8-oxoG, increases and decreases in the level of oxidation of some other DNA bases, including 8-oxoadenine, were also observed (42, 44, 48). However, this method is prone to artifactual oxidation of DNA bases, and until verified by other methods, those data should be interpreted with caution. On the basis of the available data, consumption of a single antioxidant or vitamin for weeks or months apparently provides little detectable effect on the level of oxidative DNA damage in white blood cells.

Combinations of vitamins C and E and β-carotene had a pro-
tective effect after 20 wk of supplementation but no effect after 10 wk (24) or 6 mo of supplementation (43). The length or quality of the intervention period, smoking status, or amount of antioxidants cannot explain this discrepancy. In a study that was not placebo controlled, subjects occupationally exposed to smoking had decreased 8-oxodG concentrations after 6 wk of supplementation (41). Thus, firm conclusions cannot be reached regarding whether consumption of multiple vitamins provides better protection against oxidative DNA damage than does single-vitamin supplementation.

Ingestion of natural food products produced protective effects as assessed by the comet assay (22, 23, 25), but one study of vegetable supplementation did not show an effect (34). Unfortunately, two of the positive studies were not placebo controlled (23, 25). With regard to 8-oxoG concentrations, green tea and red wine had a beneficial effect (45, 47), whereas Rao and Agarwal (40)

<table>
<thead>
<tr>
<th>Reference</th>
<th>Endpoint</th>
<th>Value for power calculation</th>
<th>n Required to detect a 50% effect</th>
<th>n In the study</th>
<th>Design score</th>
<th>Study outcome (any endpoint)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rehman et al, 1998 (42)</td>
<td>8-oxodG</td>
<td>0.24 ± 0.087 nmol/mg DNA</td>
<td>10</td>
<td>38</td>
<td>0</td>
<td>Positive</td>
</tr>
<tr>
<td>Podmore et al, 1998 (36)</td>
<td>8-oxodG</td>
<td>0.24 ± 0.06 nmol/mg DNA</td>
<td>6</td>
<td>30</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Lee et al, 1999 (37)</td>
<td>8-oxodG</td>
<td>3.38 ± 0.38 residues/10^5 dG</td>
<td>3</td>
<td>3</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Howard et al, 1998 (41)</td>
<td>8-oxodG</td>
<td>17.2 ± 11.95 pg/µg DNA</td>
<td>40</td>
<td>27</td>
<td>0</td>
<td>Positive</td>
</tr>
<tr>
<td>Thompson et al, 1999 (46)</td>
<td>8-oxodG</td>
<td>7.9 ± 6.35 residues/10^6 DNA</td>
<td>50</td>
<td>28</td>
<td>0</td>
<td>Positive</td>
</tr>
<tr>
<td>Klauung et al, 1999 (45)</td>
<td>8-oxoG</td>
<td>NR</td>
<td>NC</td>
<td>67</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Leighton et al, 1999 (47)</td>
<td>8-oxodG</td>
<td>NR</td>
<td>NC</td>
<td>42</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Duthie et al, 1996 (29)</td>
<td>SBs</td>
<td>41.0 ± 25.9 arbitrary units</td>
<td>28</td>
<td>50</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Brennan et al, 2000 (28)</td>
<td>SBs</td>
<td>16.4 ± 3.33</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>Positive</td>
</tr>
<tr>
<td>Pool-Zobel et al, 1997 (27)</td>
<td>SBs</td>
<td>4.31 ± 2.55</td>
<td>24</td>
<td>23</td>
<td>0</td>
<td>Positive</td>
</tr>
<tr>
<td>Porriti et al, 2000 (23)</td>
<td>SBs</td>
<td>0.06 ± 0.02</td>
<td>9</td>
<td>11</td>
<td>0</td>
<td>Positive</td>
</tr>
<tr>
<td>Mitchell and Collins, 1999 (22)</td>
<td>SBs</td>
<td>57.4 ± 17.4 arbitrary units</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Jenkinson et al, 1999 (30)</td>
<td>SBs</td>
<td>16.0 ± 9.4 arbitrary units</td>
<td>24</td>
<td>21</td>
<td>2</td>
<td>Positive</td>
</tr>
<tr>
<td>Torbergsen and Collins, 2000 (33)</td>
<td>SBs</td>
<td>30 ± 18 arbitrary units</td>
<td>24</td>
<td>5</td>
<td>0</td>
<td>Positive</td>
</tr>
<tr>
<td>Proteggente et al, 2000 (44)</td>
<td>8-oxodG</td>
<td>0.06 ± 0.03 nmol/mg DNA</td>
<td>17</td>
<td>20</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Rao and Agarwal, 1998 (40)</td>
<td>8-oxodG</td>
<td>21.6 ± 18.1 fmol/µg DNA</td>
<td>50</td>
<td>19</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>Chen et al, 1999 (38)</td>
<td>8-oxodG</td>
<td>2.12 ± 0.68 residues/10^5 dG</td>
<td>8</td>
<td>32</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Collins et al, 1998 (31)</td>
<td>8-oxodG</td>
<td>NR</td>
<td>NC</td>
<td>40</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Jacob et al, 1991 (39)</td>
<td>8-oxoG</td>
<td>NR</td>
<td>NC</td>
<td>8</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>Jacobson et al, 2000 (43)</td>
<td>8-oxodG</td>
<td>NR</td>
<td>NC</td>
<td>108</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Welch et al, 1999 (26)</td>
<td>8-oxoG</td>
<td>3.7 ± 1.2 residues/10^5 dG</td>
<td>40</td>
<td>9</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Tomasetti et al, 2001 (35)</td>
<td>SBs</td>
<td>100 ± 30 arbitrary units</td>
<td>7</td>
<td>6</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>Anderson et al, 1997 (27)</td>
<td>SBs</td>
<td>0.61 ± 0.35 µm</td>
<td>24</td>
<td>139</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Astley et al, 1999 (29)</td>
<td>SBs</td>
<td>0.43 ± 0.01 µm</td>
<td>2</td>
<td>9</td>
<td>3</td>
<td>Negative</td>
</tr>
<tr>
<td>van den Berg et al, 2001 (34)</td>
<td>SBs</td>
<td>89 ± 30 arbitrary units</td>
<td>9</td>
<td>21</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Pool-Zobel et al, 2000 (32)</td>
<td>SBs</td>
<td>5.1 ± 1.7</td>
<td>9</td>
<td>12</td>
<td>2</td>
<td>Negative</td>
</tr>
</tbody>
</table>

1 NR, not reported; NC, not calculated because of insufficient information on mean ± SD.
2 SBs were used for the comet assay because they are reported in all publications and probably reflect the assay variation better than do enzyme-sensitive sites or sensitivity to hydrogen peroxide or ionizing radiation because these include subtractions for the number of SBs.
3 ± SD estimated from the control group or the preintervention values.
4 Scored according to the strength of the study: 1 point for placebo groups, 1 point for parallel interventions in the placebo and antioxidant groups, and 1 point for inclusion of samples taken after the end of the intervention period. The endpoint for Value for power calculation is the number of SBs or sensitivity to hydrogen peroxide or ionizing radiation because these include subtractions for the number of SBs.
5 Estimated from graphs.
found a nonsignificant beneficial effect of lycopene-rich diets. In general, natural food products are apparently more likely to achieve beneficial antioxidant effects than are supplements. However, any conclusion should be made with caution because several of the studies were not placebo controlled. Furthermore, it is puzzling that there is no general pattern regarding the type of endpoint for which significant results occur, although it appears that the number of enzyme sites, 8-oxodG concentration, and sensitivity to hydrogen peroxide or ionizing radiation are more sensitive endpoints than the number of SBs. It is possible that various types of antioxidants exert different effects that are detected by different endpoints. The lack of a general pattern does not appear to be due to chance because chance should produce a maximum of 2 significant protective effects (given a total of 50 endpoints measured and on the basis of a two-tailed t test at a 5% significance level). A positive publication bias could be considered but is possibly not likely because short-term intervention studies typically are large studies with many endpoints from which some information eventually becomes available.

It is possible that a preventive effect of antioxidants is seen only when accompanied by an oxidative-stress situation. Oxidative stress occurs under certain physiologic conditions, eg, after exhaustive exercise; in pathologic conditions, such as immunologic diseases; or as a result of environmental factors (smoking, air pollution, or radiation of the ultraviolet A waveband) (1). Interestingly, Hartmann et al (50) showed that vitamin E supplementation (1200 mg/d) for 14 d inhibited exercise-induced SB formation. It is possible that the presence of unrecognized oxidative stress may provide a greater likelihood of detecting protective effects. It is almost impossible to identify such disguised oxidative stress in a study population, and therefore such considerations remain elusive. Although we hypothesized that beneficial effects of antioxidant supplementation would be more likely among smokers than among nonsmokers, the results of intervention studies did not confirm our hypothesis. The effect of smoking as detected by the comet assay is in itself questionable and is probably influenced by regional smoking habits and the brands smoked (51). The diet consumed also plays a role: Jenkinson et al (30) reported that the PUFA content appeared to be a strong determinant of a vitamin E–related response.

Patients with chronic inflammatory diseases (rheumatoid arthritis and systemic lupus erythematosus) and infection (hepatitis C) have been shown to have elevated concentrations of 8-oxodG in circulating leukocytes (52, 53). Diabetic patients also have markedly higher than normal amounts of oxidative DNA damage in terms of the 8-oxoG concentration and number of SBs and ENDOIII sites in leukocytes (54–56). These diseases therefore may serve as excellent models for oxidatively stressed subjects. Data from diabetic patients show conflicting effects of dietary vitamin E (29, 57) and a protective effect of a diet high in flavonoids (58). To the best of our knowledge, there have been no antioxidant intervention trials that have investigated the effects on oxidative DNA damage in patients with chronic inflammatory diseases or infections. It is conceivable that such intervention trials will prove useful for assessing the beneficial effects of antioxidants in subjects with oxidative stress related to diseases.

A critical review of short-term studies of antioxidant effects indicates that study designs need to be stronger, particularly with respect to parallel control groups and statistical power. For instance, period effects were evident in 3 studies that reported decreased 8-oxodG concentrations during the study period (38, 43, 44). In addition, seasonal variation was observed in biomonitoring studies using the comet assay (51). Mixed results were obtained from the short-term intervention studies, whereas single-dosing studies unanimously showed protective effects of antioxidants, as detected by the comet assay and measurement of 8-oxodG, in at least one blood sample taken within 24 h. An explanation for this discrepancy may be that the effects of the antioxidants are too short-lived to be detected (by chance) in one blood sample taken during the intervention.

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