How Strong Is the Evidence that Lycopene Supplementation Can Modify Biomarkers of Oxidative Damage and DNA Repair in Human Lymphocytes?1,2

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EXPANDED ABSTRACT

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Reactive oxygen and nitrogen species are produced in cells, largely as by-products of metabolic processes (1), and constantly threaten the integrity and correct functioning of cellular DNA. Several oxidant species have the capacity to produce promutagenic lesions in DNA (2), which may play an important role in the development of cancer.

Lower incidences of cancer are associated with higher consumption of fruits and vegetables (3). These foods contain relatively high amounts of components with inherent antioxidant properties, including vitamins E and C, the carotenoids, and plant polyphenols. This has led to the suggestion that dietary compounds such as lycopene augment cellular defenses, helping to protect cellular components from oxidative damage. Some purportedly protective dietary components, however, have biological activities that are not directly attributable to antioxidant effects. It is becoming increasingly evident that the carotenoids, including lycopene, may also have bioactivities capable of modifying, for example, gene expression. Thus, an alternative mechanism for the action of lycopene might be enhancement of DNA repair.

How strong the evidence is that lycopene supplementation can modify biomarkers of oxidative DNA damage and repair in human lymphocytes is determined by the reliability of DNA oxidative biomarkers, the relative importance of DNA damage compared with repair, and the validity of lymphocytes as a surrogate tissue.

Biomarkers of oxidative DNA damage

8-Oxo-2′-deoxyguanosine (8-oxo-dGuo)4 is one of the most common oxidative lesions in DNA. Its presence can result in guanine-cytosine to thymine-adenine transversion, unless repaired prior to DNA replication (4), and may consequently induce mutations. This change, however, is not a unique marker for DNA oxidative damage. There are many different oxidative DNA lesions, although few of their biological consequences have been examined in detail. This paucity of information and the inconsistent application of different methods for measuring oxidative DNA damage (e.g., detecting oxidized bases and nucleosides and strand breaks using urine as well as target and surrogate tissues) means there is still a lack of consensus both in the measurement of oxidative DNA damage, as well as its repair, and in the interpretation of its significance for human health.

Although it is perhaps natural that different laboratories should have preferred ways of expressing their results, or even different protocols for analysis, this has led to mutual incomprehension when comparing data. Similarly, the group of methods (e.g., alkaline elution, alkaline unwinding, and the comet assay), which are dependent on converting lesions to DNA breaks and measuring the frequency of these breaks, although apparently simple to use, are subject to misinterpretation. The assays can be calibrated against X-rays, but the majority consider DNA damage semiquantitatively (e.g., % DNA present in the tail, comet assay). It is, however, incorrect

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4 Abbreviations used: 8-oxo-dGuo; 8-oxo-2′-deoxyguanosine; AP, abasic site; BER, base excision repair; PBL, peripheral blood lymphocyte; SSB, single-strand break.
to assume that single-strand breaks (SSBs) relate only to DNA damage. Repair, which occurs rapidly in most cells, can cause SSBs to accumulate as an indirect indicator of ongoing repair damage. Repair, which occurs rapidly in most cells, can cause to assume that single-strand breaks (SSBs) relate only to DNA damage. Repair, which occurs rapidly in most cells, can cause SSBs to accumulate as an indirect indicator of ongoing repair damage.

**Surrogate and target tissues**

Lymphocytes are often used as surrogate tissues in experiments following response to supplementation because these cells are easily accessible from healthy volunteers and their response may reflect that in other tissues (9,10). There are, however, no experimental data to support such a relation (11); factors responsible for oxidative DNA damage or repair vary in their magnitude and effect in different tissues, and differences in metabolic rate influence the rate of oxidative damage (12) as well as the availability of resources to respond to them. For example, activities of key antioxidant enzymes, including cata-

**Biomarkers of oxidative DNA repair**

Ultimately, provided DNA repair is timely and accurate, the extent or frequency of oxidative damage is perhaps less important than DNA repair. However, although there has been considerable interest in the types and frequency of DNA lesions, repair has been largely ignored in the context of nutrition. This may be because, like oxidative damage, DNA repair is not the result of a single process. There are numerous variations, including direct repair, mismatched-base repair, base excision repair (BER), nucleotide excision repair, double-strand break repair, and damage by-pass. Each repair mecha-

**Epidemiology for lycopene and tomatoes**

In Western diets, tomatoes are usually the major source of lycopene, although there are other minor sources, such as watermelon and pink grapefruit. Lycopene, a nonprovitamin A carotenoid, provides the familiar red coloration of tomatoes, but different varieties have a unique composition, and lycopene is not the only bioactive compound present; tomatoes contain small quantities of minerals, including iron, copper, and zinc, as well as vitamins C and E, folates, and various flavonoids and phenolic acids.

Careful examination of the epidemiology demonstrates that daily tomato consumption is associated with reduced risk of cancer of the respiratory and digestive tracts, stomach, and lung; there are insufficient data to support a role in prevention elsewhere (3). There is also no difference in the protection offered by cooked tomato products compared with raw tomatoes, with the exception of prostate cancer, where consumption of cooked tomato sauces is substantially better (14). However, there is little agreement between the effects of tomatoes and lycopene. For example, in the EURAMIC study, Spanish volunteers with the lowest risk of heart attack also had the lowest plasma concentration of lycopene (15). Similarly, despite health statistics suggesting high rates of cardio-

**Studies in vitro and in vivo with lycopene supplementation**

Because reports on the effects of carotenoids are conflicting, the authors examined the similarities and differences from contiguous studies in vitro and in vivo (17). Single-cell gel electrophoresis was employed to measure the frequency of SSBs in the lymphocyte cell line MOLT-17 (as a model system) and human peripheral blood lymphocytes (PBLs) with and without oxidative challenge in the form of hydrogen peroxide (100 μmol/L, 5 min, 4°C). In this way, background levels of DNA SSBs and resistance of the DNA to oxidative challenge were assessed simultaneously, before and after sup-

The MOLT-17 human lymphocyte cell line, derived from a 5- to 9-year-old female patient with acute lymphocytic leukemia (18), was supplemented with β-carotene, lutein, or lycopene at concentrations from 0 to 8 μmol/L. Similarly, lutein, lycopene, and β-carotene, in the form of natural isolate capsules (15 mg/d for 4 wk), were used to supplement the diets of the same apparently healthy male volunteers, at the same time of year, over 3 yr in 3 separate randomized double-blind studies.

Lutein, lycopene, and β-carotene were selected because they are 3 of the most commonly consumed carotenoids in the UK: lutein from green and yellow vegetables, in particular green peas; lycopene from tomatoes and tomato products; and β-carotene from carrots and green vegetables. The range of concentrations used for supplementing the cells in vitro was selected on the basis that 1) it included typical plasma concent-

**Notes:**

- Flavonoids and phenolic acids are important for dietary nutrition. This may be because, like oxidative damage, DNA repair is not the result of a single process. There are numerous variations, including direct repair, mismatched-base repair, base excision repair (BER), nucleotide excision repair, double-strand break repair, and damage by-pass. Each repair mecha-

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isolate supplements; and 3) 8 μmol/L is twice the concentration at which antioxidant protection had previously been shown to be reduced, suggesting a shift toward pro-oxidant behavior (21).

Although supplements were used in these studies, the doses (15 mg/d) are comparable with the addition of ~150 g/d of an equivalent food source (e.g., 150 g/d of carrots, tomatoes, or green peas). Foods were not used because of confounding factors, including the presence of other compounds with bio-active potential, which could not be recreated in vitro, and personal taste preferences.

The findings of these studies are described and discussed elsewhere in detail (22). Briefly, carotenoid uptake in vitro was shown to be dose dependent. Media factors, including the presence of other compounds with bio-equivalent food source (e.g., 150 g/d of carrots, tomatoes, or lycopene, are capable of exerting 2 overlapping, but distinct effects: antioxidant protection by scavenging DNA-damage free radicals, and modulation of DNA repair mechanisms (17,22,23). Evidence from these studies and the literature since 2000 will be used to explore the strength of argument in favor of lycopene-modifying biomarkers of oxidative DNA damage and repair.

CONCLUSIONS

These data suggest that the carotenoids, including lycopene, provide protection against oxidative DNA damage, free radicals, and modulation of DNA repair mechanisms (17,22,23). Evidence from these studies and the literature since 2000 will be used to explore the strength of argument in favor of lycopene-modifying biomarkers of oxidative DNA damage and repair.

LITERATURE CITED