Human Health, Carotenoids and the Pharmanex® BioPhotonic Scanner

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Carotenoids are a family of natural fat-soluble nutrients important for antioxidant defense (Packer, 1992, 1993; Cadenas and Packer, 2002) found throughout the plant kingdom. They are responsible for the red, orange or yellow color of many fruits and vegetables, such as pineapples, citrus fruits, peaches, nectarines, persimmons, tomatoes, papaya, apricots, carrots, watermelons, pumpkins, squashes and sweet potatoes. Sometimes their presence is masked by chlorophyll, especially in dark green leafy vegetables like spinach, broccoli, collard greens, and kale.

These substances also impart color to many birds (flamingo, ibis, canary, the Egyptian vulture's brightly colored yellow head), insects (lady bug), marine animals (crustaceans, salmon) and flowers.

More than 600 carotenoids have been identified in nature but less than 50 are abundant in the human diet. Among these, five carotenoids, b-carotene, a-carotene, lycopene, lutein, and zeaxanthin are found in the blood and known to be important in human health (Khachik et al., 1992; Gerster, 1993). A large number of epidemiological and experimental studies offer strong evidence that carotenoids are nutritionally important for normal cell regeneration (Clinton and Giovannucci, 1998; Clinton, 1999), eye health (Landrum et al., 1997; Cooper et al., 1999), plus numerous other health aspects linked to unstable oxygen molecules called free radicals (Rao and Agarwal, 2000; Cadenas and Packer, 2002).

Most of the health benefits of carotenoids are associated with their action as antioxidants, that is, they protect cells and tissues from the effects of free radicals (Mortensen et al., 2001; Paiva and Russell, 1999).

Carotenoids are "sacrificial" antioxidants. In other words, carotenoid molecules are not regenerated like other antioxidants, and are degraded in the process of neutralizing free radicals or reactive oxygen species. A typical carotenoid molecule like lycopene or b-carotene is able to sustain more than 20 free radical hits by lipid radicals before it becomes completely destroyed (Tsuchiya et al., 1994). In this way, elevated tissue carotenoid levels will enhance the entire antioxidant network (Packer, 1994; Packer and Coleman, 1999) consequently reducing the danger from oxidative stress.

In addition, certain carotenoids like a and b-carotene are precursors of vitamin A. Although a daily-recommended intake (DRI) for vitamin A has been assigned (Food and Nutrition Board and Institute of Medicine, 2000) there is currently no DRI for carotenoids, including b-carotene, since they are not considered vitamins per se.

CAROTENOIDS IN SKIN

Carotenoids are not soluble in water. Therefore they are transported in blood by low-density lipoproteins (LDL) together with other fat-soluble substances like vitamin E or cholesterol. When the LDL reaches cells of the skin epidermis and dermis, carotenoids are transferred by means of lipoprotein receptors found at the surfaces of cells.

In humans, the outermost 20-30 cell layers of senescent or "dead" cells in the skin epidermis differentiate to form the stratum corneum (SC). SC cells are high in certain kinds of lipids and proteins, which act as a barrier against the effects of exposure to environmental pollutants. Carotenoids are also found in this layer, providing antioxidant and photo-protective benefits to the skin (Alaluf et al., 2002; Stahl et al., 2001).

When amounts of carotenoids in the diet are increased or carotenoid-enriched supplements like LifePak® are consumed, these substances initially accumulate in the lipoproteins in blood (Smidt et al., 1999). Their amount can be increased to a level up to 100% higher. This increase in blood carotenoids is then reflected in an increase of

carotenoid concentration in all the organs in the body, which can take up lipoproteins, including skin. Thus, the direct measurement of carotenoids on skin provides information about their levels at "site-of-action". This is a distinct advantage over measurements which depend only on carotenoids in blood plasma.

DETECTION OF CAROTENOIDS

Carotenoids can be detected by optical methods, which rely on their different spectral characteristics. However, at the skin surface high concentrations of other pigments such as melanin and hemoglobin interfere in the measurement, making accurate non-invasive carotenoid determinations impossible. Chemical methods like High Pressure Liquid Chromatography (HPLC) and Mass Spectrometry are also important techniques for detecting carotenoids, but unlike optical methods, they are invasive as tissue samples are required.

As an alternative, a new technique called the Pharmanex® BioPhotonic Scanner has been developed based on an optical method known as Resonance Raman Spectroscopy. This method has been used for many years in research laboratories for carotenoid investigations in biological systems and is described in two books published about a decade ago (Packer, 1992, 1993). The scanner measures carotenoid levels in human tissues (Emakov et al., 2001), eye (Bernstein et al., 1998) and at the skin surface (Hata et al., 2000) using optical signals, called raman signals. These signals identify the unique molecular structure of carotenoids, allowing their measurement without interference by other molecular substances.

Pharmanex® has taken the sophisticated technology of Raman Spectroscopy out of the research laboratory and developed a simple and portable instrument, the Pharmanex® BioPhotonic Scanner that can be readily used to measure human skin carotenoids. This is an enormously important development because the presence of scanners for use in field studies brings the possibility of assessing antioxidant and nutritional status to people everywhere.

The measurement of skin carotenoids by the Pharmanex® BioPhotonic Scanner is a convenient and useful indication of the body's overall antioxidant status. The reason for this is that skin carotenoid levels are a good indication of the carotenoid concentrations in blood and other tissues (Peng et al., 1995). Since carotenoids are delivered to tissues by LDL circulating in the blood, their concentrations are correlated with the amounts of the other fat-soluble antioxidants in the body such as vitamin E or co-enzyme Q. Therefore, increased levels of carotenoids reflect overall levels of antioxidant defense and diminished oxidative stress.

A study conducted by Pharmanex®, involving a large population (1,375 subjects), found compelling evidence that carotenoids are a good indicator of antioxidant status or oxidative stress (Smidt and Shieh, 2003). The study showed that people with high oxidative stress generally have low skin carotenoid levels, independent of their dietary carotenoid consumption. Specifically, the study reported that:

a. Smokers had significantly lower body defense scores (skin carotenoids) than non-smokers (13,030 vs. 19,890, respectively, $p<0.01$), independent of the number of daily fruit and vegetable servings or the calculated carotenoid consumption (using the USDA carotenoids database). These data are consistent with previous studies that reported that smoking causes oxidative stress and lowers antioxidant status (Dietrich et al., 2002; Arlberg, 2002).

b. People with habitual high sunlight exposure have significantly lower body defense scores than people with little sunlight exposure (16,446 vs. 20,085, $p<0.001$), independent of their carotenoid intake or dietary habits. Sunlight exposure is a known cause of oxidative stress and low antioxidant status, and has been demonstrated to reduce carotenoid levels (Alaluf et al., 2002; Stahl et al., 2001).

c. When analyzed by a different method based on urinary malondialdehyde excretion, an indicator of oxidative lipid damage, people with high oxidative stress had significantly lower body defense scores than people with low oxidative stress (19,392 vs. 29,590, $p<0.01$). Again, this relationship was not confounded by dietary carotenoid intakes, which were similar in both groups.

These observations provide evidence that skin carotenoids as measured by the BioPhotonic Scanner do indeed reflect the body's overall antioxidant defense status.

CONCLUSIONS

The Pharmanex® BioPhotonic skin carotenoid test provides another important indication of the body's overall antioxidant status. Its major advantage, compared to other antioxidant tests such as blood antioxidant levels or urinary oxidative damage byproducts, is related to measuring a body defense score at the skin surface where carotenoids act to protect the body from harmful stressors in the environment like ultraviolet irradiation or ozone exposure. In contrast, serum or urine measurements, which fluctuate over a wide range of values, are less reliable and they often reflect a person's intake from recent meals rather than long-term antioxidant protection. Thus measurement of skin carotenoids by the BioPhotonic Scanner is more meaningful than most other tests used to assess antioxidant status. The other major advantage is, of course, convenience and rapidity of the test. All other tests involving skin tissue sampling, blood or urine collection are inconvenient, unpleasant, complicated, require a medically trained specialist, often require several days or weeks to get the results, and of course are more expensive.

Use of the scanner technology will help Pharmanex® customers make more informed choices about lifestyle, diet and supplements for enhancing their nutrition. For these reasons, I believe introduction of the BioPhotonic Scanner by Pharmanex® is a timely and exciting development.

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