Wild Edible Plant Consumption and Age-Related Cataracts in a Rural Lebanese Elderly Population: A Case control Study

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Abstract

The Mediterranean diet is characterized by considerable diversity and high consumption of wild leafy greens which are excellent sources of antioxidants, including lutein and zeaxanthin. The latter are the only carotenoids present in the human lens and observational and intervention studies suggest they may be protective against age-related cataracts. To better understand the role of dietary diversity in general (and lutein and zeaxanthin in particular) in preventing age-related cataracts and the determinants of wild leafy greens' consumption, dietary and socioeconomic data were collected from one hundred cases and one hundred controls randomly selected from Hermel, a poor and fairly traditional Lebanese rural area. Results showed that dietary diversity and antioxidants (including but not limited to lutein and zeaxanthin) are inversely related to age-related cataracts and that wild leafy greens seem to contribute substantially to the protection against age-related cataracts by optimizing nutrient and antioxidant intake especially in those of low socio-economic status.

Résumé

Le régime Méditerranéen est caractérisé par une diversité considérable et une consommation élevée de plantes vertes sauvages, qui constituent d'excellentes sources d'antioxydants, y compris la lutéine et zéaxanthine. Ces-derniers sont les seuls caroténoïdes présents dans la lentille humaine et des études observationnelles et interventionnelles suggèrent qu'ils pourraient protéger contre la cataracte liée à l'âge. Pour mieux comprendre le rôle de la diversité alimentaire en général (et de la lutéine et zéaxanthine en particulier) dans la prévention de la cataracte liée à l'âge et les déterminants de la consommation de plantes vertes sauvages, des données socio-économiques et alimentaires ont été collectées de cent cas et cent témoins sélectionnés arbitrairement de Hermel, une région rurale pauvre et traditionnelle libanaise. Les résultats ont montré que la diversité alimentaire et les antioxydants (y compris mais non limité à la lutéine et zéaxanthine) sont inversement reliés à la cataracte liée à l'âge et que les plantes vertes sauvages sembleraient contribuer substantiellement à la protection contre la cataracte liée à l'âge en optimisant l'apport de nutriments et d'antioxydants, particulièrement chez les personnes ayant un statut socio-économique peu élevé.

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This thesis is dedicated to the memories of all the innocent victims of the 2006 Lebanese war, some of whom participated in this project. May they all rest in peace.

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1.0 INTRODUCTION

Populations living in Mediterranean countries benefit from a longer life expectancy and a lower incidence rate of chronic diseases than Northern Europeans or North Americans (Simopoulos, 2001; Schröder, 2007). Migrant studies say the Mediterranean diet and lifestyle are behind these societal differences, rather than any genetic or racial factors (James et al., 1989; Darmon & Khlat, 2001; Trichopoulou, 2004). As a matter of fact, traditional Mediterranean diets are unquestionably healthier than North European and American diets; they include a significantly large amount and variety of plant foods (such as fruits, vegetables, wild leafy greens, breads, seeds, nuts and olive oil) and thus guarantee an adequate intake of carotenoids, vitamin C, tocopherols, α-linolenic acid, various important minerals, and several possibly beneficial non-nutrient substances such as polyphenols and anthocyanins (Visioli and Galli, 2001).

Lutein and its stereoisomer zeaxanthin are members of the xanthophyll family of carotenoids. Their concentration is particularly high in dark leafy green vegetables (Mares-Perlman et al., 2002). Part of what makes these compounds unique relative to other carotenoids in humans is that they are the only carotenoids present in the macula (a small area of the retina responsible for central vision and high visual acuity) and in the lens (Bone et al., 1985; Yeum et al., 1995). Observational studies in the US have suggested that lutein and zeaxanthin may be protective against certain eye diseases such as age-related cataracts and age-related macular degeneration and intervention studies showed that lutein supplementation resulted in improved visual function in patients suffering from these eye diseases and in increased lutein levels in the eye (Alves-Rodrigues and Shao, 2004).

In Lebanon, an Eastern Mediterranean country known for its richness and diversity in wild leafy edible greens but where the diet is characterized by a heavy

reliance on refined grains as the primary source of energy (WHO, 1998), cataracts account for almost half of the causes of blindness (Mansour et al., 1997). The present project aims to lead to a better understanding of the role of dietary diversity in general, and of lutein and zeaxanthin in particular, in preventing age-related cataracts, and to determine the factors that come into play in the consumption of wild edible leafy greens, which are excellent sources of lutein and zeaxanthin as well as of other antioxidants.

2.0 LITERATURE REVIEW

2.1 THE MEDITERRANEAN DIET AND FOOD DIVERSITY

The various cultures, religious beliefs, ecologic backgrounds and historic developments around the Mediterranean basin resulted in many diets that revolved around distinct local or regional traditions but also shared a multitude of elements. Based on his observation of the food habits of some populations in the Mediterranean region, the American Ancel Keys was the first who described, in the 1960s, what was later to be known as the "Mediterranean diet" (Keys, 1980). The diet observed by Keys was based on a large variety of foods, mostly of vegetable origin, and characterized by a high consumption of fruits, vegetables, legumes, nuts, cereals and olive oil, and, on the other hand, a low consumption of meat and sausages. It's this tremendous diversity that makes the Mediterranean diet unique and is also responsible for its numerous health benefits (Simopoulos, 2001).

As a matter of fact, dietary diversity is a crucial element of a high quality diet (Johns, 2003). Not only does it guarantee an adequate intake of nutrients but also it increases their bioavailability (Kennedy et al., 2003). Moreover, dietary diversity is thought, as well, to decrease the chances of both deficiency and excess and to decrease the likelihood that any food-borne toxicant will be consumed in hazardous amounts (IFT, 1975). Recently, a 10-country study conducted by Hoddinott & Yohannes (2002) using data from Ghana, Malawi, Mali, Kenya, India, the Philippines, Mozambique, Mexico, Bangladesh and Egypt, suggested that dietary diversity could also be a useful indicator of food security (defined as energy availability). Indeed, the results indicated that in each of these ten countries, there was a positive, significant association between household diet diversity and household calorie availability per capita.

2.2 WILD LEAFY GREENS AS FOOD

The diversity of the Mediterranean diet is a result of the high plant diversity of the Mediterranean region which, in fact, houses eleven of the world's two hundred and thirty-one most important centers for plant diversity, and approximately 25,000 species, of which about half are endemic (Heywood, 1999). Factually speaking, it's the diversity of the Mediterranean region's physical and climatic conditions that makes it one of the world's major centers of plant diversity and explains why a wide variety of edible wild plants are consumed and used in a range of ways in the Mediterranean diet all year round (Simopoulos, 2001).

The people who eat wild edible plants do not usually mention them in nutritional surveys (Kabuye & Ngugi, 2001) but the use of these foods, which has evolved over the decades, has served to provide food and maintain general health among populations. In fact many of the food plants are used for both nutrition and medicine (Kabuye & Ngugi, 2001). In Jordan, Tukan et al (1998) showed different uses of common edible wild plants such as sumac (*Rhus coriara*), chicory (*Cichorium pumilum* Jacq.), Spanish thistle (*Centaurea iberica* Trev. Ex. Sprengel.), wild lettuce (*Lactuca tuberosa* Jacq.), viper's grass (*Scorzonera papposa* DC.), goat's beard (*Tragopogon coelesyriaca* Boiss.) and gundelia (*Gundelia tournefortii* L.). Interestingly, over half of these plants were consumed raw without any preliminary preparation other than cleaning and trimming. Many were also consumed as snacks thus providing important sources of nutrients as compared to some modem empty-calorie foods. Tukan et al. (1998) also highlighted the numerous ways of consuming such plants as part of salads, stews, spices or seasoning or even as hot drinks.

However, the dietary intake pattern of people worldwide is changing from a traditional diet (i.e. one containing plant and animal foods harvested from the local environment) to one containing many manufactured, processed, and

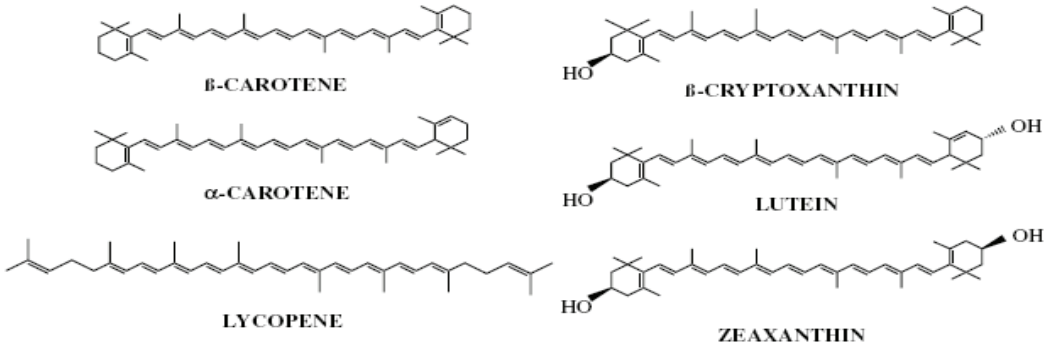
otherwise non-traditional foods (Kuhnlein & Receveur, 1996). The rapid urbanization in the region (particularly in Lebanon) might suggest a decrease in the consumption of wild edible plants and a break in the transmission of indigenous knowledge. The risks of the transition from a primarily traditional diet to one containing more market (i.e. store-bought) foods include an increase in the prevalence of chronic diseases and a decrease in the dietary intakes of some key micronutrients that are present (often in abundance) in wild edible plants (Whiting & MacKenzie, 1998). In a recent study by Batal and Hunter (2007), nutrient and food composition analyses on Lebanese wild edible plant-based dishes revealed that the latter offered a healthier alternative to increasingly common processed dishes. In fact, wild edible plants' nutritional content is superior in vitamin and mineral content to widely raised domesticated field crops (Calloway et al., 1974; Grivetti & Ogle, 2000; Farhat, 2006). In a study conducted by Humphry et al. (1993) in eastern Niger, more than eighty edible wild species were regularly used by 93% of households and contributed substantial amounts of Cu, Fe, Mg, and Zn to the diet. In Gambia, edible wild plants, especially leaf sauces prepared from edible species, are important during pregnancy and lactation (Villard & Bates, 1987). In Bangladesh, dark green leaves are major sources of pro-vitamin A (Zeitlin et al., 1992). In fact, dark leafy greens are an excellent source of carotenoids such as vitamin A precursors (such as α- and β-carotene) and xanthophylls (such as lutein and zeaxanthin) (Krinsky & Johnson, 2005).

2.3 CAROTENOIDS AND HEALTH

Carotenoids are a family of natural pigments that are widely distributed in nature and contribute to the color in plants and their fruits (Krinsky & Johnson, 2005). β-carotene is probably the best studied carotenoid because of its importance as a vitamin A precursor; however, it is only one of the approximately 600 naturally-occurring carotenoids (Krinsky & Johnson, 2005). In addition to β-

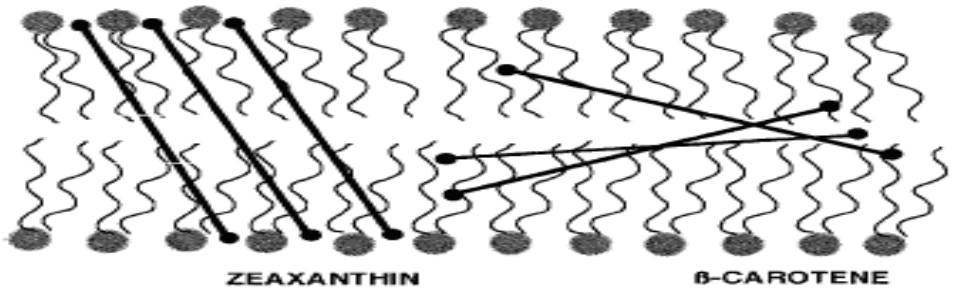
carotene, α-carotene, lycopene and lutein (**Figure 2.1**) are important carotenoid components of the human diet (Micozzi et al., 1990).

Figure 2.1: The structures of the predominant carotenoids found in human plasma (Krinsky & Johnson, 2005).



Carotenoids are mainly known for their antioxidant properties but they have also been shown to inhibit the growth of tumor cell lines, prevent bacterial mutagenesis, and modulate genotoxicity (Krinsky & Johnson, 2005). The structure of the carotenoids (especially their conjugated double bond system) gives rise to many of their fundamental properties (including their antioxidant properties) and also affects how they are incorporated into biological membranes (**Figure 2.2**). This, in turn, alters the way they interact with reactive oxygen species, so that the *in vivo* behaviour may be quite different from that seen in solution (Young & Lowe, 2001).

Figure 2.2: How the structure of the carotenoids affects their incorporation into biological membranes. β-carotene (and other carotenes such as lycopene) lies parallel with the membrane surface, deep within the hydrophobic core. In contrast, the dihydroxy carotenoid zeaxanthin entirely spans the membrane and therefore reactions with its conjugated C=C bonds are possible throughout the depth of the membrane (Young & Lowe, 2001).



The effectiveness of carotenoids as antioxidants is also dependent upon their interaction with other co-antioxidants, especially vitamins E and C (Krinksy & Johnson, 2005). Carotenoids may, however, lose their effectiveness as antioxidants at high concentrations or at high partial pressures of oxygen (Young & Lowe, 2001).

Carotenoids were implicated as protective agents, first against lung cancer and then against a variety of other chronic diseases. The results of 10 to 17 case-control studies show that a high intake of fruit and vegetables that are rich in carotenoids has been associated with decreased risk of cancer (Kinsky & Johnson, 2005). However, intervention trials employing β-carotene either have shown no preventive effect or indeed, in two cases, have enhanced the incidence of lung cancer in middle-aged male smokers and asbestos workers (Olson, 1999). On the other hand, lycopene seems to be protective against prostate cancer and plasma levels of α and β-carotene, lycopene, and lutein seem to be inversely related to ischemic stroke and myocardial infarctions (Kinsky & Johnson, 2005). In addition, lutein and zeaxanthin have been suggested to be protective against age-related macular degeneration and cataracts (Krinsky & Johnson, 2005).

2.4 LUTEIN AND ZEAXANTHIN

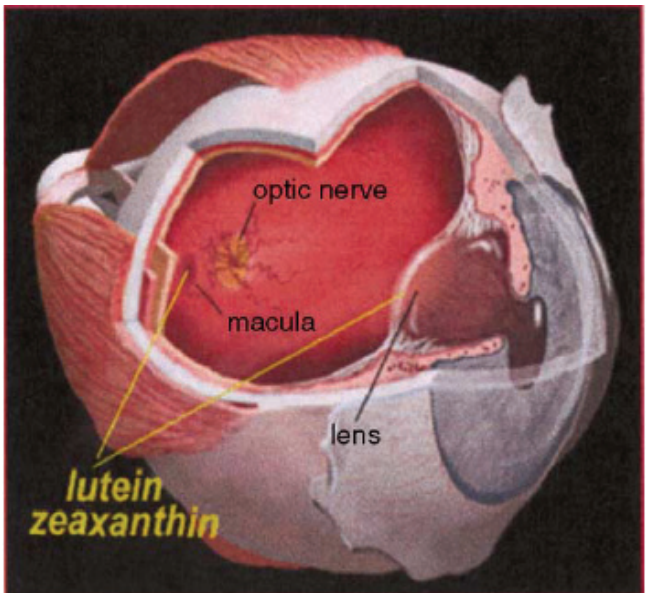
2.4.1 About lutein and zeaxanthin

Lutein and zeaxanthin are oxygenated carotenoids (xanthophylls) that consist of 40-carbon hydroxylated compounds (Pfander, 1992). Lutein was discovered in 1869 by a chemist at St. Thomas's Hospital in London called Johann Ludwig Wilhelm Thudichum who found, in parts of plants and animals, a yellow crystallizable substance, that he named 'luteine'. Zeaxanthin, on the other hand, was isolated from maize and characterized in 1929 by the Swiss biochemist Paul Karrer (Karrer et al., 1929).

Lutein and zeaxanthin cannot be synthesized by humans and must be obtained through diet. Foods that are rich in lutein and zeaxanthin include egg yolk, corn, orange juice, honeydew melon, orange pepper, and dark green leafy vegetables such as kale, spinach, collards, turnip greens, broccoli, and all kinds of wild leafy plants (Sommerburg et al., 1998; Holden et al., 1998). Recently, in 2005, Calvo compiled from the literature a huge database on the lutein composition of fresh fruits and vegetables or of fruits and vegetables submitted to different treatments. Adults on average consume around 1-2 mg of lutein per day but levels of around 3 mg per day can be easily achieved with a high fruit and vegetable diet (O'Neill et al., 2001; Mares-Perlman et al., 2001).

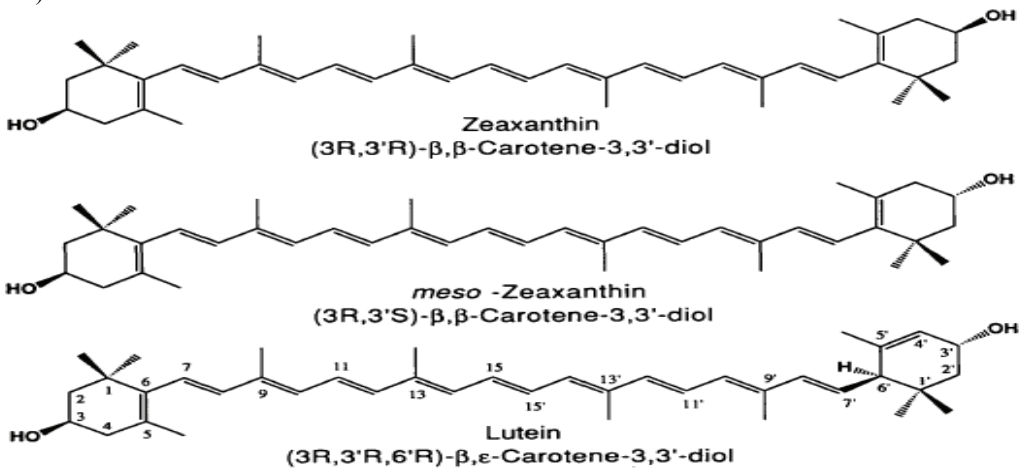
Of the 40 to 50 carotenoids typically consumed in the human diet, lutein and zeaxanthin are deposited at an up to 5-fold higher content in the macular region (**Figure 2.3**) of the retina as compared to the peripheral retina (Handelman et al., 1988). Zeaxanthin is preferentially accumulated in the foveal region whereas lutein is abundant in the perifoveal region (Bone et al., 1988).

Figure 2.3: The human eye shown in a 3D structure. Lutein and zeaxanthin are the only carotenoids present in the macula lutea, located in the mid portion of the retina, and the lens, two ocular tissues required for vision (Alves-Rodrigues & Shao, 2004).



These pigments are collectively referred to as the macular pigment; they have an intense coloration due to the extensive conjugation in their polyene chain, and give the macula its yellowish color (Landrum & Bone, 2001). Zeaxanthin is found as two isomers: 3R, 3'R-zeaxanthin and meso-zeaxanthin. Zeaxanthin and meso-zeaxanthin (**Figure 2.4**) differ in relation to the stereochemistry of the secondary hydroxyl groups at the 3' position (Bone et al., 1993).

Figure 2.4: The structures of the three major components of the macular pigment (Bone et al., 1993).



Lutein, zeaxanthin and meso-zeaxanthin represent about 36%, 18%, and 18% of the total carotenoid content of the retina but 100% of the total carotenoid content in the macula (Landrum & Bone, 2001). In addition, lutein and zeaxanthin are the only carotenoids reported to be present in eye lens (Yeum et al., 1995). What is more, there seems to be an inverse relation between macular pigment density and lens density, suggesting that the macular pigment may serve as a marker for xanthophylls in the lens. As a matter of fact, Hammond et al. (1997) have shown that higher levels of retinal lutein and zeaxanthin in the elderly were related to more transparent lenses. One interpretation of these results is that higher retinal lutein and zeaxanthin may predict higher lenticular lutein and zeaxanthin, which could lead to a more protected lens resulting in increased transparency.

2.4.2 Absorption, transport, and bioavailability

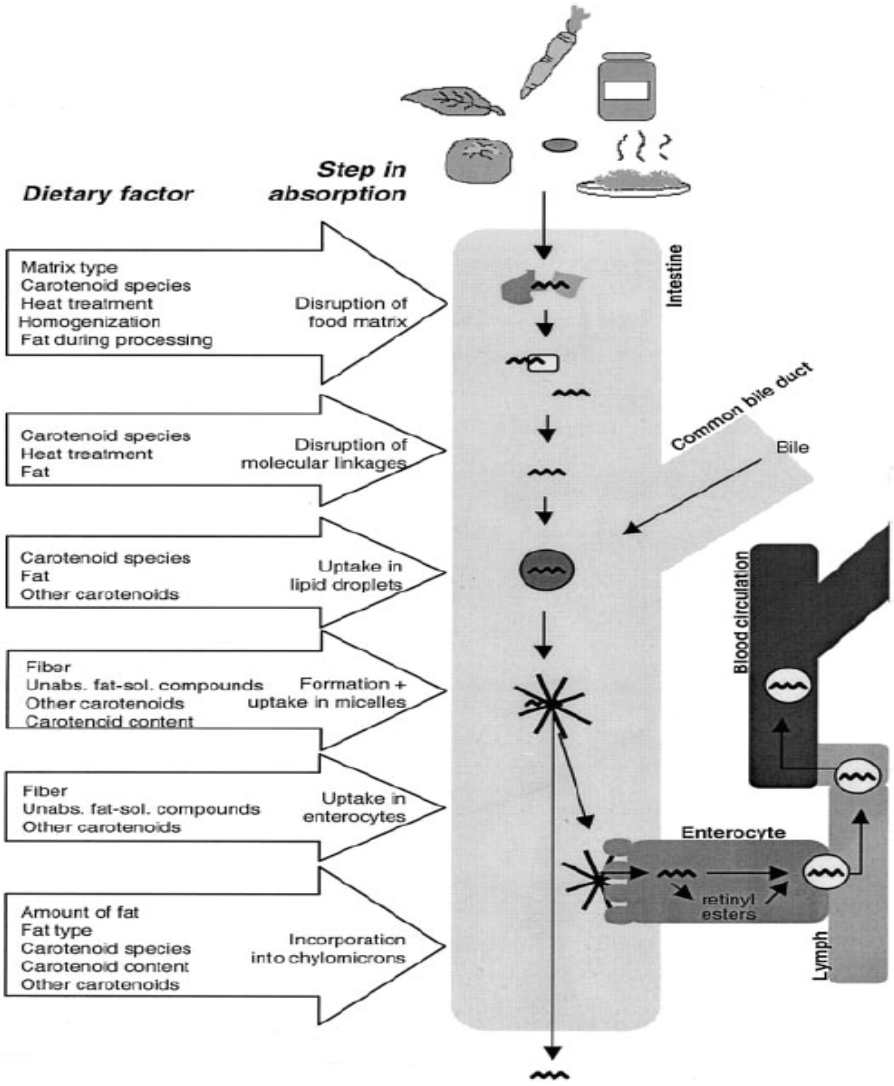
During the digestion process (**Figure 2.5**), dietary lutein or zeaxanthin esters are hydrolyzed in the lumen of the small intestine prior to uptake by the mucosa (Wingerath et al., 1995). Their bioavailability is important since they are not synthesized by humans and since most of the studies performed reported that they had a low bioavailability in general (Calvo, 2005).

Many factors influence lutein or zeaxanthin bioavailability (**Figure 2.5**). One important factor is solubility (Amar et al., 2003). Other factors include food source, dietary fat, food preparation and processing (Van het Hof, 1999), and interaction with other carotenoids (Tyssandier et al., 2002). The most likely mechanism for this interaction is competition between the carotenoids for incorporation into the mixed micelles during digestion. Another possible mechanism could be that there is competition between carotenoids for uptake and metabolism in the enterocytes or for incorporation into the chylomicrons.

Furthermore, Van het Hof et al. (1999b) found that the bioavailability of lutein varies substantially among different vegetables. Riso et al. (2003) for example, found that the ingestion of both spinach and broccoli raises the serum levels of lutein a few hours after eating but that after the ingestion of spinach, serum lutein concentration remains high until 80 hours after ingestion. The above studies have determined the plasma response of carotenoids after supplementation with vegetables or fruits and compared it with the response to supplementation with pure carotenoids. "Relative carotenoid bioavailability" is obtained by dividing the plasma responses that are induced by vegetables or fruit consumption and corrected for differences in intake by those induced by pure carotenoid supplementation (Van het Hof et al., 2000). The relative bioavailability of lutein from a diet supplemented with a variety of vegetables is much greater than that of ß-carotene (67 and 14%, respectively) (Van het Hof et al., 1999a). The same was

found for the relative bioavailability of lutein and ß-carotene from spinach (45 and 5.1%, respectively) (Castenmiller et al., 1999).

Figure 2.5: Steps of carotenoid absorption and dietary factors that affect carotenoid absorption (Van het Hof et al., 2000).



Also, the amount of fat in the diet affects carotenoids' bioavailability. For example, the amount of fat required for optimal intestinal uptake of lutein esters is higher than the amount of fat required for optimal uptake of vitamin E and α- and β-carotene (Roodenburg et al., 2000). On the other hand, sucrose polyester fat substitutes (Olean, olestra) can lower concentrations of serum lutein and other

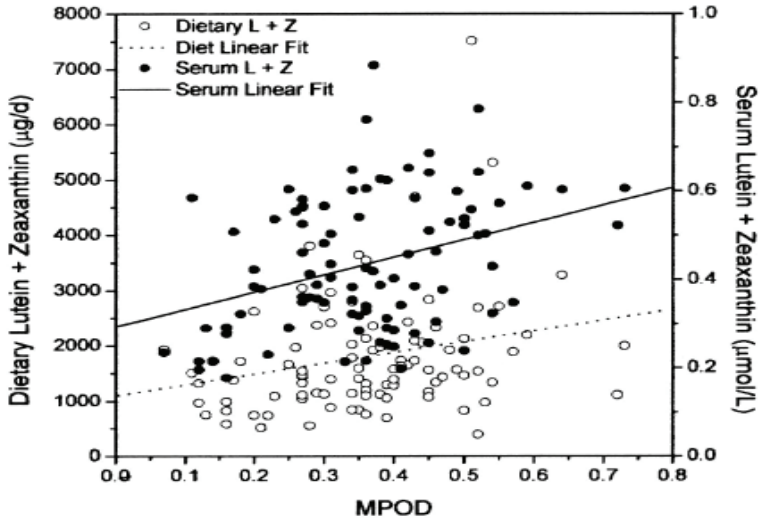
carotenoids (Westrate &Van het Hof, 1995) but apparently do not affect possible markers of disease risk in humans (Broekmans et al., 2003). Cholesterol-lowering phytosterol and stanol products also reduce carotenoid concentrations, albeit to a lesser extent (Weststrate & Meijer, 1998; Plat &Mensik, 2001).

Once in the human blood stream, high-density lipoproteins (HDL) are the major carriers of lutein and zeaxanthin while carotenes are preferentially carried by low-density lipoproteins (LDL) (Clevidence &Bieri, 1993). Every 10% increase in estimated dietary intake of lutein and zeaxanthin was associated with a 2.4% increase in serum lutein concentration (Rock et al., 2002). Other determinants of serum lutein and zeaxanthin include cholesterol and lipoprotein status, metabolic status, body composition, smoking and BMI (Rock et al., 2002). Dietary lutein may be converted to mesozeaxanthin (Bone et al., 1993) or serve as a precursor for the very high concentrations of zeaxanthin found in the primate fovea (Bone et al., 1997).

Lutein and zeaxanthin-rich diets and serum lutein and zeaxanthin positively contribute to the macular pigment status in the retina (Burke et al., 2005). The macular pigment's optical density increases with increasing dietary intake and serum levels of lutein and zeaxanthin (**Figure 2.6**).

Figure 2.6: Macular pigment optical density versus dietary (µg/d) and serum (µmol/L) lutein and zeaxanthin.

Subjects' macular pigment optical density was positively associated with both dietary ($r = 0.237$, $P = 0.02$, $n = 96$) and serum ($r = 0.342$, $P = 0.0006$, $n = 95$) lutein and zeaxanthin (Burke et al., 2005).



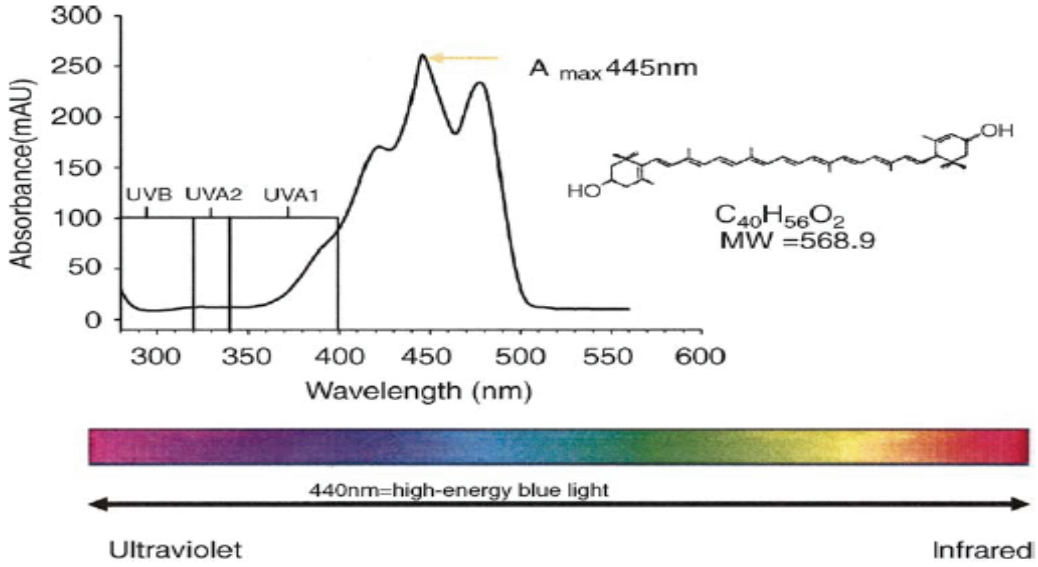
In fact, human macular membranes could be a rich source of specific binding proteins for lutein and zeaxanthin once these two reach the eye. In a recent report, xanthophyll-binding proteins (XBP) were partially purified and isolated from the human macula and retina and it was shown that lutein and zeaxanthin bind specifically to these proteins (Yemelyanov et al., 2001). Available evidence further suggests that adipose tissue and retina may compete for dietary lutein (Johnson et al., 2000).

2.4.3 Mechanism of action

As a highly vascularized tissue possessing a high concentration of polyunsaturated fatty acids, the macula is particularly susceptible to oxidative damage (Beatty et al., 1999). Lutein and zeaxanthin are believed to protect the retina in two ways. Firstly, they are thought to play a role in the protection against light-dependent damage. By absorbing blue-light, they protect the underlying macular photoreceptor cell layer from light damage (possibly initiated by the formation of reactive oxygen species during a photosensitized reaction). The high absorptivity of lutein and zeaxanthin in the inner retina functions as an efficient filter for blue light. This filtering effect reduces chromatic aberration and short wavelength sensitivity (Reading & Weale, 1974). It is also believed to be responsible for the relative preservation of foveal short-wavelength cone sensitivity with age (Haegerstrom-Portnoy, 1988).

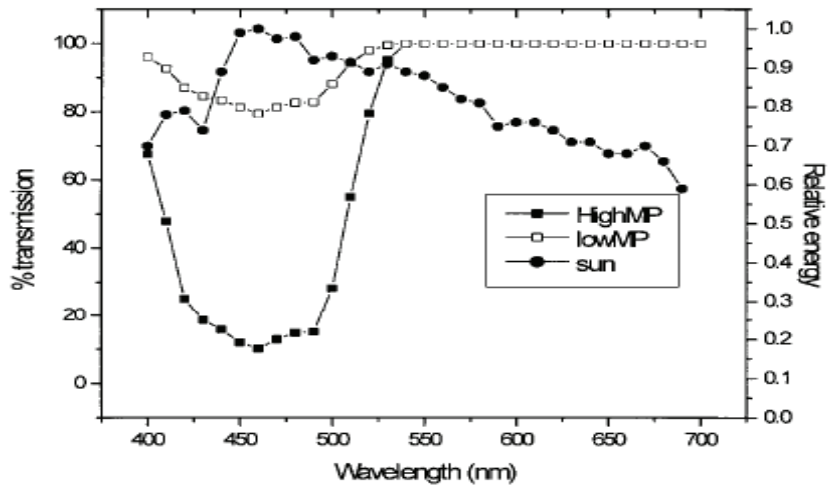
Blue light is the highest energy form of visible light, and is known to induce photo-oxidative damage by generating reactive oxygen species (Alves-Rodrigues & Shao, 2004). The absorbance spectrum of macular pigment peaks at 460 nm (Pease et al., 1987) but lutein has an absorption maximum of about 445nm (**Figure 2.7**) and zeaxanthin of about 451 nm (Schmidt-Erfurth, 2005).

Figure 2.7: Absorption spectrum for lutein. Lutein's peak absorption is at 445 nm which corresponds to the blue wavelength of light in the visual spectrum (Alves-Rodrigues & Shao, 2004).



It is estimated that macular pigments reduce the blue light intensity normally by 40% (Landrum et al., 1997). However, if sufficiently dense, they only allow 10% of 460nm light (**Figure 2.8**) to reach the vulnerable outer segments of the foveal cones (Wyszecki & Stiles, 1982).

Figure 2.8: Macular pigment optical density and light transmission. A double Y plot showing subjects with high v/s low macular pigment (left ordinate) plotted against (right ordinate) the relative energy of midday sunlight (correlated color temperature of 6500°K) derived from tabular data provided in Wyszecki and Stiles (1982), (Hammond et al., 2001).



In the outer retina, oxygen partial pressure is so high that it results in a high rate of blue light-induced singlet oxygen formation (Schmidt-Erfurth, 2005). In fact, one of the ways light damages the retina is by generation of free radicals that lead to peroxidation of membrane lipids (Johnson, 2002). Direct oxidation products of lutein and zeaxanthin have been reported there, indicating that these carotenoids do act as antioxidants in the macula (Khachik et al., 1997). Actually, it is hypothesized that lutein and zeaxanthin are able to quench free radicals and to react with the peroxy radicals that are involved with lipid peroxidation (Landrum & Bone, 2001). The inhibition of lipid peroxidation is desirable in the retina because of the high concentration of polyunsaturated fatty acids in the photoreceptor membranes (Beatty et al., 2000). On the other side of the coin, lutein may also prevent cellular damage in certain forms of cardiovascular disease (Dwyer et al., 2001), stroke (Ascherio et al., 1999), and cancer (Bidoli et al., 2003) by quenching singlet oxygen or neutralizing photosensitizers.

Lutein and zeaxanthin do not protect only the retina from oxidative damage. They also protect the lens since, within the lens, around 74% of lutein and zeaxanthin are located between the epithelium and the cortex, area where oxygen might be expected to stress the lens the most strongly (Yeum et al., 1999).Thus, these antioxidant pigments may help prevent oxidation of epithelial lipids, which is an important etiological factor in cataract development (Bhuyan & Bhuyan, 1984). In fact, in 2004, Chitchumroonchokchai et al. provided the first data demonstrating that lutein and zeaxanthin decrease UVB-induced lipid peroxidation and attenuate the activation of the stress signaling pathways in human lens epithelial cells. Trevithick-Sutton et al. (2006) decided to further investigate the possibility that lutein and zeaxanthin exerted their protective effect by scavenging free radicals by examining their effect on superoxide and hydroxyl radicals. Both lutein and zeaxanthin scavenged the hydroxyl radicals more effectively than superoxide radicals, zeaxanthin being the most powerful hydroxyl radical scavenger. The mechanism of hydroxyl radical scavenging could occur, according to Trevithick-Sutton et al. (2006), via bond formation between the

hydroxyl radical and one of the double bonds in lutein and zeaxanthin. Lutein, containing only 10 conjugated double bonds, compared to the 11 conjugated double bonds of zeaxanthin (**Figure 2.4**), scavenges hydroxyl radicals less effectively.

2.5 EVIDENCE SUPPORTING A PROTECTIVE ROLE FOR LUTEIN AND ZEAXANTHIN IN TWO COMMON EYE DISEASES OF AGING

2.5.1 Lutein and zeaxanthin and age-related cataracts

Age-related cataracts are the most common type of cataracts. Cataracts are ocular opacities, partial or complete in one or both eyes, on or within the lens. They are divided into three subtypes based on location (nuclear, cortical and posterior subcapsular). These opacities are caused by precipitation of oxidatively damaged proteins in the lens of the eye, often resulting in impaired vision or blindness (Bron et al., 2000).

Age is the most significant risk factor for late-onset cataract and the relationship between aging and lens optical density has been studied extensively (Coren & Girgus, 1972; Pokorny et al., 1987; Sample et al., 1988; Hammond et al., 1997). Smoking (DeBlack, 2003), diabetes (Klein et al., 1998; Saxena et al., 2004; Hennis et al., 2004) and UV light exposure (McCarty & Taylor, 2002) have all also consistently been found to be associated with age-related cataracts. Dark iris color alters the effect of UV radiation that reaches the eye (possibly by raising the temperature of the lens and thus increasing molecular degradation and age-associated increases in lens optical density) and thus was found too to be directly related to age-related cataracts (Hammond et al., 2000).

On the other hand, body mass index seems to be related in a U-shaped manner to age-related cataracts even though the nature of the relationship between

body mass index and age-related cataracts has not been fully elucidated yet (Klein et al., 1998; Jacques et al., 2003; Glynn et al., 1995; Weintraub et al., 2002; Hiller et al., 1998). Women seem to have higher rates of cataract across racial groups, even when adjusting for women's greater longevity, probably because, by nature, they have more body fat than men do (Hennis et al., 2004; McCarty et al., 1999). In addition, dietary fat and serum lipids also seem to contribute to cataract risk since linoleic acid, linolenic acid, and other unsaturated, cis-configurated fatty acids, were found to have a cytotoxic effect on lens epithelial cells (Iwig et al., 2004) while eicosapentaenoic and docosahexaenoic acid had a protective effect (Lu et al., 2005).

Finally, studies examining the association between age-related cataracts and myopia (McCarty et al., 1999; Mukesh et al., 2006), corticosteroids use (Carnahan & Goldstein, 2000; Toogood et al., 1993), exogenous estrogen use (Mukesh et al., 2006; Younan et al., 2002) and genetics (Sekine et al., 1995; Alberti et al., 1996; Okano et al., 2001; Maraini et al., 2003) have yielded conflicting results.

Experimental studies in vivo

Many experimental animals do have lenses but since the levels of lutein and zeaxanthin in the lenses of these animals are not known, and since there is no evidence that dietary lutein or zeaxanthin influence the levels of these carotenoids in the lenses of those experimental animals, there were no studies on cataracts in experimental animals (Mares-Perlman et al., 2002). The only experimental evidence that lens carotenoids in adult vertebrates can be manipulated by dietary supplements was provided two years ago by Dorey et al. (2005). Quails fed with a diet supplemented with a high dose of zeaxanthin had significantly higher lens zeaxanthin than quails fed with a diet with no or a lower dose of zeaxanthin (p <0.0001). Unfortunately, the study did not examine the impact of supplementing

the diet with lutein on lens carotenoids and lens lutein was not affected by dietary supplementation with zeaxanthin ($p \geq 0.18$).

Epidemiological evidence

A few studies have specifically examined the relationship between lutein and zeaxanthin and cataract risk. Chasan-Taber et al. (1999) noted that US women who had the highest intake of lutein and zeaxanthin had a 22% decreased risk of cataract extraction compared with those in the lowest quintile (RR: 0.78; 95% CI: 0.63, 0.95; *p*- trend = 0.04). In men with higher intakes of lutein and zeaxanthin but not of other carotenoids, Brown et al. (1999) also noted that there was a lower risk of cataract extraction: men in the highest fifth of lutein and zeaxanthin intake had a 19% lower risk of cataract relative to men in the lowest fifth (RR: 0.81; 95% CI: 0.65–1.01; *p*-trend=0.03).

Moreover, in 1995, a retrospective study by Mares-Perlman et al. of 1919 participants in the Beaver Dam Study, found that women in the highest quintile category of lutein intake ten years prior to study enrollment (median 949 μg/day) had a 27% lower prevalence of cataracts (OR: 0.73; 95% CI: 0.5–1.06; *p*-trend=0.02) than women in the lowest quintile category of lutein intake (median 179 μg/day). Of the lutein-rich foods examined, only spinach was found to be inversely associated with cataract formation.

In 1999, after five years of follow-up, Lyle et al. (1999a) found that members of the Beaver Dam cohort who were in the highest quintile category of lutein intake 10 years before baseline (median 1,245 μg/1000 kcal) were 50% less likely to develop nuclear cataracts (RR: 0.5; 95% CI: 0.3–0.8; *p*-trend=0.002) than those in the lowest quintile category of intake (median 298 μg/1000 kcal). As in the first study, consumption of spinach and other dark leafy greens at baseline was inversely associated with risk of nuclear cataract. In addition, an inverse association between serum lutein concentrations and risk of nuclear cataract was

observed in people aged more than 65 years (OR: 0.3; 95% CI: 0.1–1.2; *p*-trend=0.15), although this association was not statistically significant (Lyle et al., 1999b).

As a matter of fact, the Melbourne Visual Impairment Project, an Australian population-based prevalence study of eye disease that included both residential and nursing home populations, found that nuclear cataract was the only type of cataract to be significantly associated with dietary intake of lutein and zeaxanthin (Vu et al., 2006). An inverse association was observed between high dietary intake of lutein and zeaxanthin and the prevalence of nuclear cataract (OR: 0.58; 95% CI: 0.37-0.92; *p*-trend = 0.023 and OR: 0.64; 95% CI: 0.40-1.03; *p*-trend =0.018 for those in the top quintile of crude and energy-adjusted lutein and zeaxanthin intake, respectively). Bearing this in mind, Gale et al. had found in 2001 that, after adjustment for age, gender, and other risk factors, the risk of nuclear cataract was lowest in people with the highest plasma concentrations of α-carotene (OR: 0.5; 95% CI: 0.3-0.9; *p*-trend = 0.006) or β-carotene (OR: 0.7; 95% CI: 0.4-1.4, *p*-trend = 0.033). Plasma concentrations of lutein were only significantly associated with a lower risk of posterior subcapsular cataract (OR: 0.5; 95% CI: 0.2-1.0; *p*-trend = 0.012) and plasma concentrations of zeaxanthin did not seem to be inversely associated with any type of cataract.

On the other hand, a prospective analysis of the Nurses Health Study cohort reported that the rate of cataract surgery was associated with lower intakes of lutein-rich foods such as spinach and other green vegetables (Hankinson et al., 1992). Consumption of spinach and other greens at least five times per week compared to less than once a month resulted in a 47% lower risk for cataract extraction during up to eight years of follow-up (RR: 0.53; 95% CI 0.38–0.73; *p*-trend=0.001).

In a double-blind study involving dietary supplementation with lutein (15 mg/d, 3 times/wk for up to 2 years, n = 5), α-tocopherol (100 mg/d, 3 times/wk (n

= 6) or placebo (n = 6) in patients with cataracts, visual performance (visual acuity and glare sensitivity) improved in the lutein supplemented group only (Olmedilla et al., 2003). As a matter of fact, a recent study reported that a high dose combination of antioxidants (vitamins C and E, β-carotene, and zinc) had no significant effect in the development or progression of cataract (Age-Related Eye Disease Study Research Group, 2001b). Similarly, The Roche European–American Anticataract Trial (REACT), a randomized placebo-controlled 3-year trial, concluded that a mixture of oral antioxidant micronutrients (b-carotene, 18 mg/d; vitamin C, 750 mg/d; vitamin E, 600 mg/d) only produced a small deceleration in progression of age-related cataract (Chylak et al., 2002).

These studies suggest, despite differences in study design, case definition and exposure measurement, that dietary lutein and zeaxanthin play a role in cataract prevention and that spinach and other dark leafy greens, the most concentrated sources of dietary lutein and zeaxanthin, are most consistently associated with protection against cataract.

2.5.2 Lutein and zeaxanthin and age-related macular degeneration

Age-related macular degeneration is the principal cause of blindness in the elderly population in the USA and the Western world (Mozaffarieh et al., 2003). It is a degradation of the central portion of the retina which includes the macula. There are two types of age-related macular degeneration: “wet” (or neovascular) and “dry” (or atrophic) (The Macular Degeneration Partnership, 2007). It is possible to experience both types at the same time, in one or both eyes. In “wet” age-related macular degeneration, tiny unhealthy blood vessels grow under the retina and often break and leak, causing loss of vision. Conversely, in “dry” age-related macular degeneration, the most common type, there is a breakdown or thinning in the macula of the retinal pigment epithelial cells (RPE) which are light sensitive and contain hundreds of photoreceptors. The death or degeneration of these cells is called atrophy (hence, the name atrophic age-related macular

degeneration) and reduces one's central vision and can affect color perception. Generally, the damage caused by the "dry" form is not as severe or rapid as that of the "wet" form but can, over time, cause profound vision loss.

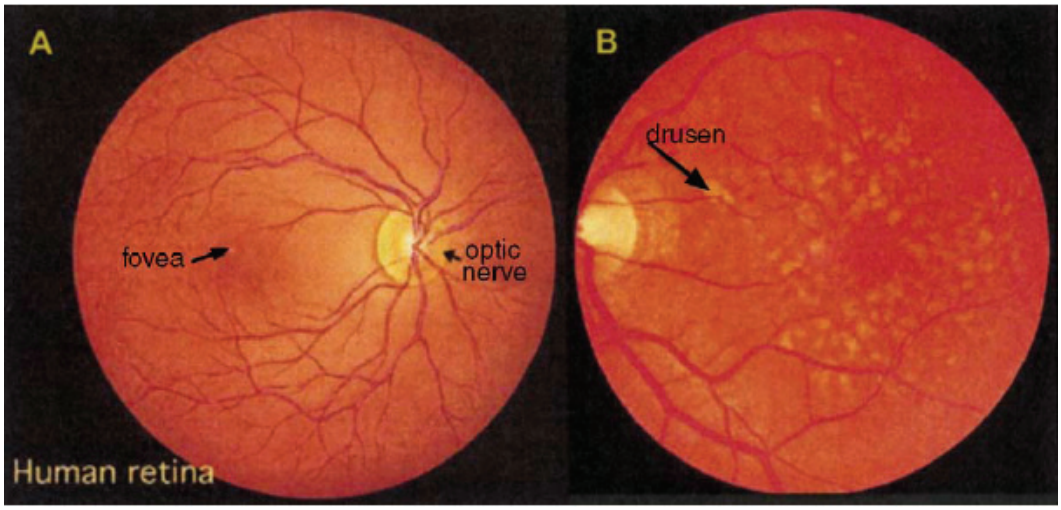
Aside from age (McCarty et al., 2001) and family history (Silvestri et al., 1994; Heiba et al., 1994; Klein et al., 2001b), smoking (McCarty et al., 2001; Thornton et al., 2005) has been the most consistent factor associated with the prevalence and, to a lesser extent, the incidence of age-related macular degeneration. High body mass index was also positively associated with age-related macular degeneration (Klein et al., 2001a) but, oddly enough, the latter was inversely related to blood lipid levels (Hyman et al., 2000; Klein et al., 2003a). In fact, persons with low serum high-density lipoprotein (HDL) cholesterol or high low-density lipoprotein (LDL) cholesterol were more likely to have a lower prevalence and incidence of age-related macular degeneration (according to Malek et al. (2003), because of the downregulation of LDL receptors that occurs in the retinal pigment epithelium, subsequent to high plasma cholesterol). Nonetheless, there are neither epidemiologic nor clinical trial data showing an association of lipid-lowering with a reduction in risk of age-related macular degeneration.

On the other hand, observational studies linking age-related macular degeneration to systemic hypertension (Hyman et al., 2000; Klein et al., 1993; The Eye Disease Case Control Study Group, 1992), atherosclerosis (Vingerling et al., 1995a; Klein et al., 1993), exogenous estrogen use (The Eye Disease Case Control Study Group, 1992; Klein et al., 1994), statin use (McGwin et al., 2003; Klein et al., 2003b), gender (Vingerling et al., 1995b; Klein et al., 1992), UV light exposure (Klein et al., 1993; The Eye Disease Case Control Study Group, 1992) and iris color (Hammond et al., 1996; Nicolas et al., 2003; Khan et al., 2006) have given conflicting results.

Experimental studies in vivo and in vitro

Only primates have the anatomical feature of a macula; thus it is not physically possible to study the role of carotenoids in macular degeneration in experimental animals such as rodents for example, who do not have a macula. In monkeys fed diets devoid of plant pigments for several years, levels of these pigments in the macula disappear and retinal abnormalities that resemble age-related degenerative changes in humans (drusen accumulation **Figure 2.9**) appear (Malinow et al., 1980).

Figure 2.9: Funduscopic matter of the human eye and retina. (A) Normal, healthy human retina and (B) Retina from a patient advanced AMD. Irregularly shaped white spots represent drusen, a collection of oxidatively damage cells and proteins. Adapted from the University of Utah Webvision. by Alves-Rodrigues & Shao (2004).



Xanthophyll profiles in quail mimic those in primates: Quail retina, like the primate macula, is dominated by cone photoreceptors and concentrates lutein and zeaxanthin. Moreover, retinas of aged quails exhibit the characteristics of aged primate retinas including age-related loss of photoreceptors (Fite & Bengston, 1989). Preliminary studies indicate an inverse correlation between the level of zeaxanthin in quail retina and light-induced retinal cell death (Dorey et al., 1997; Thomson et al., 2002).

Epidemiological evidence

There has been a substantial interest in the role of diet and nutrition in age-related macular degeneration. A large case control study by the Eye Disease Case Control Study (EDCCS) Group compared the fasting serum samples of 615 controls to 421 patients recently diagnosed with the less common, but more severe, neovascular or wet form of age-related macular degeneration (The Eye Disease Case-Control Study Group, 1993). Those with lutein/zeaxanthin concentrations ≥0.67 μmol/L were 70% less likely to have age-related macular degeneration than those with concentrations ≤0.25 μmol/L (OR: 0.3; 95% CI: 0.2–0.6; *p*-trend=0.0001). In a subsequent study by Seddon et al. (1994), among 356 cases and 520 controls, those in the highest quintile of carotenoid intake had a 43% lower risk of developing age-related macular degeneration compared to those in the lowest quintile. From all the carotenoids ingested, the strongest association with protection from age-related macular degeneration was found for lutein and zeaxanthin. Subjects who were in the highest quintile for their intake of lutein and zeaxanthin had a 57% lower risk of advanced age-related macular degeneration (OR: 0.43; 95% CI: 0.2–0.7, *p*-trend<0.001) compared to those in the lowest quintile and subjects in the highest quintile for consumption of spinach had an 86% lower odds ratio of advanced age-related macular degeneration.

In 2001, Bone et al. obtained donor eyes from age-related macular degeneration patients and control subjects, and measured the actual concentrations of lutein and zeaxanthin in the area including and surrounding the macula. Within the area most closely surrounding the macula, those subjects possessing the highest concentration were 82% less likely to have age-related macular degeneration relative to those with the lowest concentration.

However, not all studies have found an association between serum carotenoids or macular pigment concentrations and protection from age-related macular degeneration. For example, a case-control study that used a sample of

167 case-control pairs selected from the population of Beaver Dam Eye Study found no such association for lutein or zeaxanthin (Mares-Perlman et al., 1995). Another study by VandenLangenberg et al. (1998) that followed subjects from the Beaver Dam cohort who were free of late stage age-related macular degeneration at baseline for five years found no significant association between specific macular lesions and lutein and zeaxanthin intake, either 10 years before study enrollment in the study or at baseline. Similarly, a small case control study by Sanders et al. (1993) in the United Kingdom found no significant differences between the mean plasma lutein concentrations of 65 patients with age-related macular changes and the lutein concentrations of 65 control subjects.

On the other hand, recent studies suggest that lutein supplementation may improve visual function in age-related macular degeneration patients. Falsini et al. (2003) evaluated the influence of short-term antioxidant supplementation of vitamin E, nicotinamide, and 15 mg/d lutein for 180 days to age-related maculopathy patients and control subjects on retinal function by recording focal electroretinograms. A significant increase in amplitude change of the focal electroretinograms was reported in patients and controls with antioxidant supplementation. However, the specific effects of any one component could not be assessed. The Lutein Antioxidant Supplementation Trial (LAST), a double-blind, randomized, placebo-controlled study, supplied 90 males with atrophic age-related macular degeneration with either 10 mg of lutein, 10 mg of lutein plus a broad spectrum formula containing antioxidants/vitamins/minerals, or placebo for one year (Richer et al., 2004). Lutein and antioxidant supplementation resulted in positive effects on visual function, including improved contrast sensitivity, glare recovery, and visual acuity (Richer et al., 2004).

Differences in the range of lutein and zeaxanthin exposures and/or the severity of age-related macular degeneration may partly explain the inconsistent findings of these results (Moeller et al., 2000). Additional epidemiologic studies that demonstrate consistency of associations across populations and further

evaluate the strength of the association of dietary intake of lutein and zeaxanthin with a reduced risk of developing cataracts or age-related macular degeneration are needed. Evidence is also needed to prove that the findings are specifically due to the intake of lutein and zeaxanthin rather than other aspects of diet or lifestyle that are more common in people who eat diets rich in these carotenoids (Marles-Perlman et al., 2002). Thus, the evidence to support the possibility that lutein and zeaxanthin promote eye health is emerging but currently insufficient.

2.6 OTHER ANTIOXIDANTS AND AGE-RELATED CATARACTS & MACULAR DEGENERATION

2.6.1 Other antioxidants and age-related cataracts

Dietary antioxidants accumulated by the lens include not only lutein and zeaxanthin but also vitamin C and vitamin E (Taylor et al., 1991; Yeum et al., 1999). These antioxidants along with β-carotene have been found collectively or individually to influence the risk for cataract. In fact, higher dietary or serum levels of vitamin E (Vitale et al., 1993; Leske et al., 1998; Rouhiainen et al., 1996; Mares-Perlman et al., 2000) and vitamin C (Taylor et al., 1991, 2002; Mares-Perlman et al., 2000; Jacques et al., 2001) have been associated with a reduced risk for cataract in both cross-sectional and longitudinal studies. These associations are reinforced by evidence from intervention studies which showed that cataract risk was reduced in subjects whose diet was supplemented with tocopherol (Leske et al., 1998; Mares-Perlman et al., 2000), ascorbate (Taylor et al., 1991; Mares-Perlman et al., 2000; Taylor et al., 2002) or a mixture of ascorbate, tocopherol, and β-carotene (Chylack et al., 2002).

Unfortunately, risk for cataracts has not been consistently associated with tocopherol, ascorbate, or β-carotene. As a matter of fact, the Age-related Eye Disease Study, a prospective study of 4629 subjects, found no reduction in the 7-

year risk for age-related cataracts of any type in those given multivitamins or supplemental ascorbate, tocopherol, β-carotene and zinc (Age-Related Eye Disease Study Group, 2001). Neither plasma ascorbate nor β-carotene influenced cataract risk in the Baltimore Longitudinal Study on Aging (Vitale et al., 1993) and plasma tocopherol was not associated with risk for cataracts in England (Gale et al., 2001), or lens optical density in the Netherlands (Berendschot et al., 2002). Moreover, tocopherol supplements had no effect on the incidence of cataracts or rate of cataract extraction in Australia (McNeil et al., 2004) or on the prevalence of cataracts in middle-aged Finnish smokers (Teikari et al., 1997).

On the other hand, higher dietary levels of certain carotenoids such as carotenes and lycopene, which are not present in the human lens, were associated with reduced risk for cataracts in several studies (Yeum et al., 1995; Bates et al., 1996; Bernstein et al., 2001). As a matter of fact, intakes of α-carotene, β-carotene, and total carotenoids were related to risk for posterior subcapsular cataracts in American women (Taylor et al., 2002) and higher plasma concentrations of lycopene, and α- or β-carotene were respectively associated with a reduced risk for cortical and nuclear cataracts in English subjects (Gale et al., 2001). In addition, β-carotene supplements were found to reduce excessive risk for cataracts among smokers by ≈ 25% (Christen et al., 2003) and supplementation with a mixture of β-carotene and vitamin E significantly slowed the increase in density of cataractous lenses of American subjects (Chylack et al., 2002). Nonetheless, β-carotene and vitamin E had no effect on lens optical density of English subjects (Chylack et al., 2002) or on risk of cortical, nuclear or posterior subcapsular cataracts in Finnish smokers (Teikari et al., 1997).

2.6.2 Other antioxidants and age-related macular degeneration

As with cataracts, there is a substantial body of evidence on diet and age-related macular degeneration but no consistent findings to support a protective

role for dietary antioxidants other than lutein and zeaxanthin in delaying age-related macular degeneration. Case control and population-based studies suggest, however, that important associations do exist between micronutrients with antioxidant properties and age-related macular degeneration.

In fact, the most compelling of these associations were found in the Eye Diseases Case Control Study (1993) where there was a progressive decrease in the risk of age-related macular degeneration with increasing serum levels of carotenoids and increasing antioxidant index (a composite score based on serum carotenoids, selenium, vitamins C and E). Similarly, in the Baltimore Longitudinal Study of aging, high levels of plasma α-tocopherol or a high antioxidant index (constructed from plasma ascorbic acid, α-tocopherol, and β-carotene) were protective for age-related macular degeneration (West et al., 1994). In the National Health and Nutrition Examination Survey, which was one of the first studies to evaluate the role of nutrition in ophthalmic disorders, the frequency of fruits and vegetables rich in vitamin A consumed was negatively correlated with age-related macular degeneration (Goldberg et al., 1988). In addition, interestingly enough, subjects who were in the lowest quintile for serum lycopene were twice as likely to have either wet or dry age-related macular degeneration in the Beaver Dam Eye Study (Mares-Perlman et al., 1995a). More recent small studies also observed positive associations between age-related macular degeneration status and serum vitamin E (Belda et al., 1999) or dietary intakes of antioxidants (Snellen et al., 2002).

On the other hand, no association was found between age-related macular degeneration and serum β-carotene, serum α-tocopherol, dietary antioxidants, or oral zinc in the Blue Mountain Eye Study (Smith et al., 1997; Flood et al., 2002). On the contrary, quite surprisingly, increased serum vitamin C was associated with an increased risk for age-related macular degeneration (Flood et al., 2002). No significant associations between vitamin supplementation and risk of age-related macular degeneration were found either in the large Physicians Health

Study I (Christen et al., 1999), and in the POLA study, plasma tocopherol levels were only weakly negatively associated with age-related macular degeneration (Delcourt et al., 1999). It is also noteworthy to mention that even though a trial by Newsome et al. (1988) showed that zinc-treated subjects had significantly less visual loss than the placebo group after a follow-up of 24 months, a subsequent trial by Stur et al. (1996) using a similar study design has failed to substantiate these findings.

The above-mentioned case control and population-based studies have raised intriguing hypotheses and have thus provided the basis for the multiple interventional studies that have been carried out. AREDS, the largest and most robust randomized controlled trial concerning supplements to date, found a significant reduction in the rate of progression of age-related macular disease with the use of a multivitamin-multimineral combination supplement made of the vitamins C and E, ß-carotene, and zinc (Age-Related Eye Disease Study Research Group, 2001a) but the results of other trials with these antioxidants and age-related macular degeneration were disappointing. A small trial by Kaiser et al. (1995) investigated the effect of Visaline (vitamin C, vitamin E, β-carotene, and buphenine) on the progression of age-related macular degeneration and failed to show significant differences in any of the parameters measured. Likewise, the Vitamin E intervention in the Cataract and Age-related macular degeneration Trial (VECAT) concluded that daily supplementation with 500 IU of vitamin E does not prevent the development or progression of age-related macular degeneration (Tikellis et al., 1999). Moreover, the α-tocopherol β-carotene (ATBC) trial, a population-based controlled study examining the effect of long-term supplementation with vitamin E and β-carotene on age-related macular degeneration status, found no statistically significant differences between those who were receiving the supplements and those who were not (Teikari et al., 1998).

2.7 STUDY RATIONALE

Cataract is one of the major causes of blindness throughout the world (WHO, 2004). In the US, the prevalence of cataract increases from around 5% at age 65 to around 40% for persons older than 75 (Klein et al., 1992). In less developed countries like India (Chandrashekhar et al., 2007), China (Xu et al., 2006) or Kenya (Mathenge et al., 2007), cataracts are more common and develop earlier in life than in more developed countries.

There are around 6.3 million blind and around 22 million people with visual impairment in the Middle Eastern Region according to the WHO (2005). In Lebanon, 0.6 % of the population is blind and 3.9% suffers from low vision (Mansour et al., 1997). Cataracts are responsible for 41.3% of the causes of blindness and macular degeneration for 3.8% (Mansour et al., 1997). Lutein and zeaxanthin have been shown to have a protective role against both age-related cataract and age-related macular degeneration; however, since the prevalence of macular degeneration in Lebanon is very low, the present study will only investigate the effect of the intake of lutein and zeaxanthin on the incidence of age-related cataracts. Many factors contribute to the low prevalence of macular degeneration: a shorter lifespan of individuals living in eastern Mediterranean countries than that of those living in the United States or Europe, an early onset of cataract which may prevent light-related damage to the macula, and the fact that the retinal pigment epithelium of dark skinned individuals may protect against macular damage (Tabbara, 2001). Several epidemiologic studies have also shown that dark-colored irises decrease the risk for developing age-related macular degeneration (Hammond et al., 1996; Nicolas et al., 2003), even though some studies found no significant association between iris color and the incidence of age-related macular degeneration (Khan et al., 2006).

Moreover, most of the evidence on the relationship between lutein and zeaxanthin and the eye diseases of aging (i.e. age-related cataract and age-related

macular degeneration) is based on epidemiological studies done in the United States. The particularity of the present study is that it will try to establish a link between dietary diversity in general, and green vegetable consumption in particular, and an eye disease of aging (age-related cataract) in a developing country where the diet is characterized by a heavy reliance on refined grains as the primary source of energy (WHO, 1998), and where cataracts account for more than 40% of the causes of blindness (Mansour et al., 1997). Furthermore, it hopes to promote dietary diversification in Lebanon and the consumption of dark leafy vegetables (esp. wild leafy edible plants) to combat age-related cataracts and improve overall health.

2.8 STUDY OBJECTIVES

1. Assess the relationship between lutein and zeaxanthin intake and age-related cataracts:

    a. Determine if high or low intake of lutein and zeaxanthin is associated with the development of age-related cataracts.

    b. Determine whether the intake of lutein and zeaxanthin is related to the intake of wild edible leafy greens.

    c. Determine the main source of lutein and zeaxanthin in the diet of people with a high intake of lutein and zeaxanthin.

2. Determine the factors that come into play in wild edible leafy greens' consumption:

    a. Develop an in-depth understanding of the role of indigenous knowledge about wild leafy greens' identification and collection in increasing wild leafy greens' consumption and improving ocular health.

    b. Determine the socio-economic factors that affect the consumption of wild edible leafy greens.

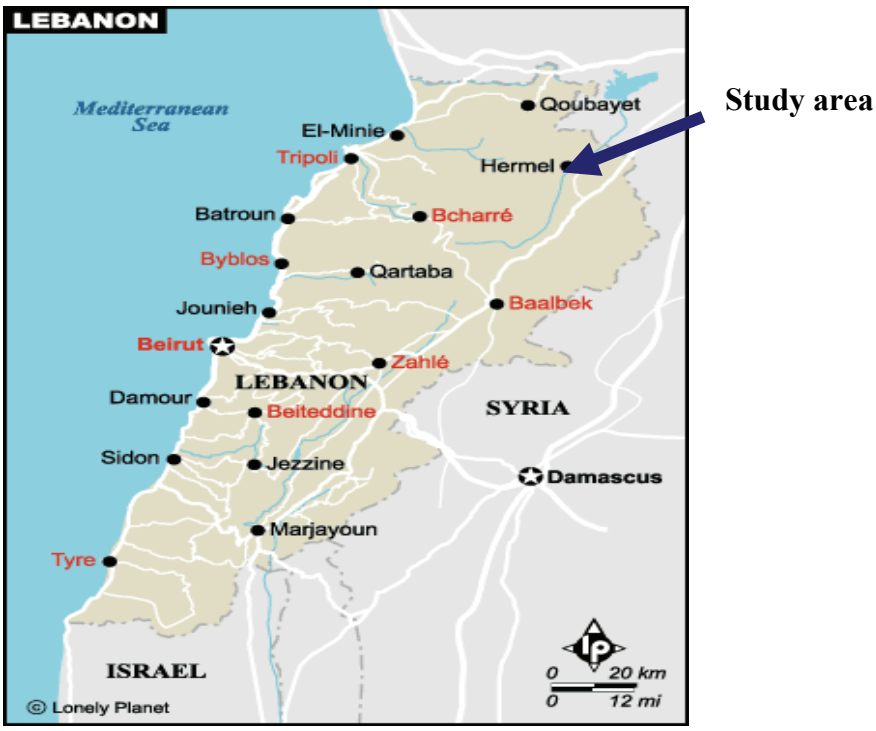
3. Determine whether dietary diversity is linked with the development of age-related cataracts.

3.0 SUBJECTS AND METHODS

3.1 STUDY AREA AND POPULATION

The study was part of a project conducted by the American University of Beirut in collaboration with McGill University and funded by IDRC. The project, which started in December 2004, principally aimed to improve dietary diversity in poor communities in Lebanon by promoting the consumption of wild edible plants. Dr. Malek Batal, the project's principal investigator, suggested that the study takes place in one of the project's study sites, Hermel. Hermel is a poor, ethnically homogeneous and fairly traditional rural area of around 100,000 inhabitants situated in the northeast of Lebanon in the Bekaa Valley, 134 km from the capital city of Beirut (**Figure 3.1**). Due to its location in between the two Lebanese mountain chains, the area receives little precipitation and is semi-arid (US Library of Congress).

Figure 3.1: Map of Lebanon (Lonely Planet).



To be included in the study, the participants had to be older than 45 and native and resident of the villages where the study was conducted. They were located through a local eye clinic and through random walk. For each participant with age-related cataract or who has had age-related cataract extraction there was a control of the same age, gender and region, with no history of diabetes, who has visited an eye clinic for a check-up but has been tested negative for any eye disease.

Approval for the study was granted from the "Research Ethics Committee" of McGill's faculty of Agricultural and Environmental Sciences and from the "Institutional Review Board" of the American University of Beirut's Faculty of Medicine. The study was explained in detail to all potential participants and their voluntary consent was solicited. We insured that all subjects thoroughly understood the informed consent (which was translated to Arabic) before signing it. Participants who could not read or write gave their consent verbally and a member of their family was asked to sign for them. Upon completion of data collection, preliminary study results were to be presented to the community at a town meeting but the unfortunate circumstances that took place in summer 2006 in Lebanon prevented that from happening. Nevertheless, such a meeting is to be organized in the near future.

3.2 STUDY DESIGN

A case-control study design was employed to explore the relation between the intake of carotenoids (and especially lutein and zeaxanthin) along with diet diversity and age-related cataract risk. A nutritionist and 3 local community members with previous training in administering questionnaires conducted the interviews in groups of two. A meeting was held with the interviewers before the start of the interviews and a training module was followed to ensure a common

understanding of the goals and objectives of the project and the meaning of the questions in the questionnaire.

3.3 SAMPLING PROCEDURE

Our sample size consisted of 100 cases and 100 controls. To reduce sampling bias, cases and controls were both to be selected from a local eye clinic. However, since not enough patients were visiting the clinic and were going instead to nearby Syrian clinics which were much cheaper, 98% of the participants were eventually selected by random walk. The Hermel district comprises the town of Hermel (capital of the district) and a collection of small surrounding villages. Sampling started in the town of Hermel where the survey team spun a bottle or a pen at every crossing to determine the direction to continue and visited the households in the chosen direction in search of patients with age-related cataracts or who have had age-related cataract extraction (who if found and selected were matched with people living in the neighborhood of the same age and gender who have been tested negative for any eye disease during the last eye check-up they had). Each group of interviewers was to recruit 10 participants per day. If not enough participants could be recruited in a certain neighborhood, another direction was randomly chosen until the required number of participants was obtained.

3.4 INTERVIEWS

A nutritionist and 3 local community members collected all dietary, demographic, and anthropometric data. All spoke Arabic fluently and understood the dialect particular to the area. Almost all interviews were conducted in the homes of the participants (apart from those conducted in the eye clinic). Data were collected during the month of June 2006 when the wild edible leafy greens'

season was coming to its end. The questionnaires were translated to Arabic and each interview took around 30 to 45 minutes. Two questionnaires were administered to each of the participants. The first collected socio-demographic data and included a 7-day semi-quantitative food frequency questionnaire. The second consisted of a 3-month food frequency questionnaire that was administered a week after data collection for the first questionnaire was done. This strategy was used to familiarize participants with filling up food frequency questionnaires at first in order to collect later on more accurate data on food consumption in general and the consumption of wild leafy greens in particular (during their growth season which goes from the end of February until the end of May approximately).

3.4.1 Socio-demographic assessment

Socio-demographic data was gathered from the participants during the first interview. As a matter of fact, the first questionnaire collected demographic information, data on socioeconomic conditions, and data on indigenous knowledge about wild plant identification and collection.

3.4.2 Dietary assessment

Two structured semi-quantitative food frequency questionnaires (a 7-day and a 3-months food frequency questionnaire respectively) separated by a one-week interval were used to describe patterns of food intake. Calibrated local utensils were available at each interview to help participants describe quantities of food eaten. The food-frequency questionnaires included a total of 82 food items each but were not exhaustive: they were composed of local foods rich in lutein and zeaxanthin, local foods rich in other carotenoids (such as β-carotene and lycopene), and staple foods. Food items were divided into 9 categories: breads and cereals, fruits, vegetables, beans, milk and dairy products, meats and eggs, fats and oils, drinks, and wild edible leafy greens. The participants were asked to

recall their usual frequency of consumption of a given food item and the amount consumed daily, weekly, or monthly.

The CANDAT Nutrient Calculation System (2005) was used to process the dietary data obtained from the food frequency questionnaires. A user file was created to include Lebanese foods (such as labneh, kishek, etc.) and local wild edible leafy greens. The lutein and zeaxanthin contents of the foods were estimated from existing nutrient composition databases (USDA National Cancer Institute Carotenoid Food Composition Database; Calvo, 2005; O'Neill et al., 2001) and incorporated into the program. When data on wild leafy greens was not available, an average value from the databases was used.

3.4.3 Diversity indexes

For this study, two indexes of diversity were used: Food Variety Score (FVS) and Dietary Diversity Score (DDS) (Ogle et al., 2001). FVS was obtained from a count of the number of foods consumed during the last 3 months and was calculated for the total number of foods consumed, foods rich in lutein and zeaxanthin, foods rich in retinol activity equivalents (RAE), foods rich in β-carotene, foods rich in α-tocopherol, and foods rich in vitamin C. Food items that were considered to be rich in lutein and zeaxanthin were those that contained 500 μg or more of lutein and zeaxnthin per portion size: this amount constituted 25% of the mean daily intake of lutein and zeaxanthin for our study population and approximately 50% of the mean daily intake of lutein and zeaxanthin according to the literature. Thirty-two food items out of 82 were thus considered to be rich in lutein and zeaxanthin. Using a similar logic, the 32 food items highest per portion size in any other nutrient studied (retinol activity equivalents (RAE), β-carotene, α-tocopherol, and vitamin C) were considered to be rich in that particular nutrient. However, only 29 food items out of the possible 82 contained non-negligible amounts of α-tocopherol per portion size; they were the ones considered to be "rich" in α-tocopherol.

DDS was obtained from a count of food groups consumed over the last 3 months. Two DDS were created. Both had a total number of 8 food groups and included starches, fruits, vegetables, legumes, dairy products, meats, fats and oils. The first DDS however also comprised leafy greens while the second comprised wild leafy greens.

3.4.4 Anthropometry

Age was obtained from the national identity card of each participant. Height and mid-upper arm circumference were measured using a measuring tape and were recorded to the nearest one-tenth centimeter. When standing height could not be measured (because of postural problems or confinement to bed), knee-height was used to estimate it with equations derived from North American people and based on age and gender (Chumlea et al., 1994). Weight was measured with a digital scale and was recorded to the one-tenth kilogram. Body Mass Index (BMI) was computed by weight (kg)/ height (m^2).

3.5 DATA ANALYSES

Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows version 13.0 (SPSS Inc., 1989-2004). Nutrient intakes were not normally distributed, therefore the Mann-Whitney U test, the nonparametric equivalent of Student's t-test, was performed to test the differences between cases and controls. Spearman's rho correlation coefficient was used to assess the degree of association between the intake of lutein and zeaxanthin and that of wild leafy greens. In addition, a stepwise multiple linear regression was used to determine the best predictors of the consumption of wild edible leafy greens. The data on the intake (in grams) of wild leafy greens was not normally distributed and had therefore to be log-transformed before the regression could be run. The independent variables entered into the regression were age, gender, duration of

residence in Hermel, marital status, education level, occupation, socio-economic status, smoking, and source of knowledge on wild leafy greens. In the present study, $p<0.05$ was used to indicate statistical significance.

4.0 RESULTS

4.1 SAMPLE CHARACTERISTICS

Participants were between the ages of 45 and 90 years (**Table 4.1**). Both male and female participants were approximately the same age (around 67 years old on average). Most participants were illiterate, had always lived in Hermel, and classified themselves as "poor". In fact, more than three quarters were unemployed, homemakers, or retired. Two thirds of the participants were married. Around three quarters, of both cases and controls, had dark-colored irises. More than half were cigarette or hookah smokers at one point in their lives. However, more cases than controls worked in agriculture, had a family history of age-related cataracts, and a high level of psychological stress.

Mean BMI was 31 and wasn't significantly correlated with age. BMI ranged from 18 to 47.6 kg/m^2 with 1% having a BMI below 18.5 kg/m^2, the cut-off point used by the WHO to identify chronic energy deficiency, and 81% having a BMI over 25 kg/m^2. MUAC and BMI were statistically significantly positively correlated ($\rho = 0.479$, $p<0.01$); however, only about 10% of the participants were above the 85^{th} MUAC percentile and surprisingly, as many as 10% were below the 10^{th} MUAC percentile, using the age and gender-specific MUAC percentiles for older men and women examined in the NHANES III (Kuczmarski et al., 2000). Both cases and controls had approximately the same BMI and MUAC on average.

4.2 NUTRIENT INTAKES AND AGE-RELATED CATARACTS

The distribution of the average intakes per day over a period of three months for lutein and zeaxanthin, β-carotene, Retinol Activity Equivalents (or vitamin A), vitamin C and α-tocopherol is described in quantiles along with the

mean (**Table 4.2**). Values are compared to FAO/WHO recommendations. Participants at the 25th percentile met the recommended intakes for lutein and zeaxanthin. Participants at the 50th, 75th and 90th percentiles met the recommendations for vitamin C, vitamin A and β-carotene, respectively. However, the 90th percentile value for α-tocopherol was below the recommended value.

The distribution of the average energy intakes per day over a period of three months by the participants (71% women) was divided according to their BMI and gender and is described in quantiles along with the mean (**Table 4.3**). The 90th percentiles values for both normal weight and overweight men and women were below the FAO/WHO/UNU recommended values.

Controls had a significantly higher intake of energy, lutein and zeaxanthin, β-carotene, vitamin A and vitamin C than cases (Mann-Whitney U test, $p<0.001$) (**Table 4.4**). On the other hand, intake of α-tocopherol was not significantly different between cases and controls (Mann-Whitney U test, $p>0.3$).

Average intake of lutein and zeaxanthin per day over a period of three months (μg) was significantly correlated with the intake of wild leafy greens ($\rho = 0.332$, $p<0.01$) using Spearman's rho correlation coefficient (as the distribution of both variables was markedly skewed). However, the major contributors of lutein and zeaxanthin did not include wild leafy greens (**Table 4.5**). The three main sources of lutein and zeaxanthin in the diet of subjects with a high intake of lutein and zeaxanthin (N=67) were in fact spinach, Swiss chard and parsley. The main sources of lutein and zeaxanthin did not differ between the diet of cases (N=26) and controls (N=41) that had a high intake of these two nutrients (**Table 4.6**).

4.3 WILD LEAFY GREENS' CONSUMPTION

More than 90% of both cases and controls consumed wild leafy greens. Around 40% collected them and 90% of those learned about wild leafy greens and their identification and collection from their parents (**Table 4.7**).

The average intake of wild leafy greens (g) during their growing season (a period of three months) was very similar between cases and controls (**Table 4.7**). Roughly 18% of the variation in intake could be explained by a simple model equation that was derived from linear regression analysis (**Table 4.8**):

$$I = 0.15\ K + 0.29\ A + 0.18\ U + 0.69$$

where I is the log-transformed intake (g) of wild leafy greens and K, A and U represent the following predictors: knowledge on wild leafy greens acquired from parents, agriculture as present or past occupation and unemployment, respectively.

4.4 DIVERSITY SCORES

Controls had a significantly higher Food Variety Score (FVS) than cases and consumed a significantly higher number of food items rich in lutein and zeaxanthin, β-carotene, vitamin C and α-tocopherol than cases did over a period of three months (Mann-Whitney U test, $p<0.001$) (**Table 4.9**). However, the number of food items rich in vitamin A consumed did not significantly differ between cases and controls (Mann-Whitney U test, $p>0.5$).

Likewise, there was no significant difference between the Dietary Diversity Scores of cases and controls neither when leafy greens (Mann-Whitney U test, $p>0.6$) nor when wild leafy greens (Mann-Whitney U test, $p>0.06$) were considered as part of the 8 groups we divided our food items into (**Table 4.10**).

Table 4.1: Sample characteristics.

	Cases					Controls				
	n	Min.	Max.	Mean	SD	*n*	Min.	Max.	Mean	SD
Gender										
Female	71					71				
Male	29					29				
Age	100	45	90	66.5	11.4	100	45	90	66.5	11.4
Body Mass Index	100	18	45.5	29.9	6.1	100	20	47.6	31.8	6.4
Mid-upper arm circumference (cm)	100	21	41	30.7	4.2	100	22	40	31.1	3.6
Iris color										
Dark (brown or black)	73					76				
Smoker (at any point in life)	47					53				
Socio-economic status										
Poor	77					62				
Marital status										
Married	54					63				
Education level										
Illiterate	74					62				
Place of residence										
Resident of the area since birth	79					86				
Occupation										
Agriculture (present or past)	35					15				
Unemployed, homemaker,or retired	82					77				
Family history of age-related cataracts	33					11				
High level of stress (>5 on a scale from 1 to 10)	79					57				

Table 4.2: Distribution of nutrient intakes compared to the FAO/WHO vitamin recommended nutrient intakes (FAO/WHO, 2002).

Nutrient	FAO/ WHO	Mean	Percentile				
			10th	25th	50th	75th	90th
Lutein and Zeaxanthin (μg)	1000*	2681.7	954	1763	2619	3314	4117
Vitamin A (μg)	600	663.7	205.5	360	578.5	939.2	1164.8
β-carotene (μg)	3600	2806	1065.3	1801.25	2588	3494.5	4457.2
Vitamin C (mg)	45	48.3	22.4	33.6	45.7	57.7	69.4
α-tocopherol (mg)	7	1.9	0	1	2	3	3.9

* Estimated usual intake of lutein and zeaxanthin. There are to date no recommended intakes for lutein and zeaxanthin.

Table 4.3: Distribution of energy intakes for males (N=58) and females (N=142) according to their weight and compared to the FAO/WHO/UNU recommended energy intakes for older adults and elderly (FAO/WHO/UNU, 2001).

		FAO/ WHO/ UNU	Mean	Percentile				
				10th	25th	50th	75th	90th
Energy (kcal)	Males	3048 - 1700†	928.5*	617.6	726.5	871	1001.3	1454.2
		3465 - 2294‡	986.3**	644.4	804.5	942	1166	1345.6
	Females	2441 - 1382†	819.8*	475.3	667.5	821	952	1170
		3032 - 1748‡	998.8**	632.4	807.5	1014	1164.5	1276.4

† TEE measured with DLW for men and women 40 to 90 yo of normal weight (BMI of 18.5-25 kg/m^2).

‡ TEE measured with DLW for 40 to 90 yo overweight men and women (BMI > 25 kg/m^2).

* Males (N=16) and females (N=22) with a BMI of 18.5-25 kg/m^2. Only two female participants had a BMI below 18.5 kg/m^2.

** Males (N=41) and females (N=121) with a BMI higher than 25 kg/m^2.

Table 4.4: Comparison of the average 3-month intake of energy (kcal), lutein and zeaxanthin (µg), vitamin A (µg), β –carotene (µg), vitamin C (mg) and α - tocopherol (mg) between cases and controls. The values are given as the mean and the standard deviation.

	Cases	**Controls**	***p*-value***
Energy (kcal/d)	872.6 ± 233.6	1069.3 ± 251.0	<0.001
Lutein and Zeaxanthin (µg/d)	2268.2 ± 1163.3	3095.3 ± 1460.2	<0.001
Vitamin A (µg/d)	550.9 ± 407.1	776.5 ± 396.7	<0.001
β-carotene (µg/d)	2277.0 ± 1423.8	3335.0 ± 1687.2	<0.001
Vitamin C (mg/d)	43.5 ± 23.4	53.0 ± 25.4	<0.001
α-tocopherol (mg/d)	1.9 ± 1.2	1.8 ± 1.4	0.33

* *p*-value of Mann-Whitney U's test for differences in intake between cases and controls.

Table 4.5: Main sources of lutein and zeaxanthin in the diet of subjects (N=67) with a high intake of lutein and zeaxanthin.

Food item	Lutein and zeaxanthin (µg/d)
1. Spinach	652.5
2. Swiss chard	556.2
3. Parsley	415.4
4. Eggplant [a]	397.1
5. Mint [a]	243.6
6. Wheat grains [a]	186.7
7. Chicory	162.7
8. Lettuce	153.3
9. Amaranth [b]	148.2
10.Faba beans [a]	120.0

[a] Calvo, M.M. (2005). Lutein: a valuable ingredient of fruit and vegetables. *Critical Reviews in Food Science and Nutrition, 45*, 671-96.

[b] Su, Q., Rowley, K.G., Itsiopoulos, C., & O'Dea, K. (2002). Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: green leafy vegetables, figs and olive oil. *European Journal of Clinical Nutrition, 56,* 1149-1154.

Table 4.6: Comparison of the main sources of lutein and zeaxanthin in the diet of cases (N=26) and controls (N=41) with a high intake of lutein and zeaxanthin.

Cases		Controls	
Food item	**Lutein and zeaxanthin (µg/d)**	**Food item**	**Lutein and zeaxanthin (µg/d)**
1. Spinach	574.1	**1**. Spinach	703.3
2. Eggplant [a]	492.2	**2**. Swiss chard	619.0
3. Parsley	464.1	**3**. Parsley	385.2
4. Swiss chard	457.4	**4**. Eggplant [a]	336.8
5. Mint [a]	219.6	**5**. Mint [a]	259.0
6. Wheat grains [a]	206.2	**6**. Chicory	178.5
7. Amaranth [b]	179.7	**7**. Wheat grains [a]	174.4
8. Lettuce	145.6	**8**. Lettuce	158.2
9. Chicory	139.6	**9**. Amaranth [b]	130.1
10.Faba beans [a]	111.2	**10**.Faba beans [a]	125.7

[a] Calvo, M.M. (2005). Lutein: a valuable ingredient of fruit and vegetables. *Critical Reviews in Food Science and Nutrition, 45*, 671-96.

[b] Su, Q., Rowley, K.G., Itsiopoulos, C., & O'Dea, K. (2002). Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: green leafy vegetables, figs and olive oil. *European Journal of Clinical Nutrition, 56,* 1149-1154.

Table 4.7: Collection and consumption of wild leafy greens.

	Cases			Controls		
	n	Mean	SD	*n*	Mean	SD
Collect wild leafy greens	38			41		
Ethnobotanical knowledge acquired from parents	33			35		
Consume wild leafy greens	92			98		
Wild leafy greens consumed (g/d)*	100	10.1	7.3	100	10.1	8.4

* $p>0.63$ using Mann-Whitney U's test to compare cases to controls.

Table 4.8: Predictors of the consumption of wild leafy greens.

Predictors*	**Unstandardized coefficients**		**Standardized coefficients**	**95% confidence interval for β**	
	β	Std. Error		Lower Bound	Upper Bound
(Constant)	0.69	0.05		0.58	0.79
Ethnobotanical knowledge acquired from parents	0.15	0.05	0.22	0.06	0.24
Occupation: Agriculture (present or past)	0.29	0.07	0.42	0.15	0.43
Unemployed	0.18	0.06	0.28	0.06	0.30
R^2	0.173				

* Intake of wild leafy greens (g) was not normally distributed and had therefore to be log-transformed before the regression could be run.

Table 4.9: Comparison of the Food Variety Scores (FVS) and the number of food items rich in lutein and zeaxanthin, vitamin A, β-carotene, vitamin C and α-tocopherol consumed over a 3-month period between cases and controls. The values are given as the mean and the standard deviation.

	Max.	**Cases**	**Controls**	***p*-value***
Food Variety Score (FVS)	**82**	41.1 ± 8.8	47.8 ± 9.4	<0.001
Number of food items rich in lutein and zeaxanthin	**32**	14.3 ± 4.5	16.9 ± 4.4	<0.001
Number of food items rich in vitamin A	**32**	15.5 ± 3.8	15.1 ± 3.9	0.56
Number of food items rich in β-carotene	**32**	12.8 ± 3.7	15.2 ± 3.5	<0.001
Number of food items rich in vitamin C	**32**	11.7 ± 3.5	14.0 ± 3.7	<0.001
Number of food items rich in α-tocopherol	**29**	11.3 ± 3.0	13.3 ± 3.4	<0.001

* *p*-value of Mann-Whitney U's test for differences in food variety between cases and controls.

Table 4.10: Comparison of the Dietary Diversity Scores (DDS) between cases and controls. Foods were divided in the following eight groups: starches, fruits, vegetables, legumes, dairy products, meats, fats and oils, and leafy greens or wild leafy greens. The values are given as the mean and the standard deviation.

	Max.	**Cases**	**Controls**	***p*-value***
Dietary Diversity Score (DDS) – leafy greens	**8**	7.97 ± 0.2	7.98 ± 0.1	0.66
Dietary Diversity Score (DDS) – wild leafy greens	**8**	7.89 ± 0.3	7.96 ± 0.2	0.06

* *p*-value of Mann-Whitney U's test for differences in food diversity between cases and controls.

5.0 DISCUSSION

INTERPRETATION OF RESULTS

Since cases and controls were matched according to age, there was no age difference between the two groups. Males and females as well as smokers and never-smokers seemed to have the same risk of having age-related cataracts whereas in the literature, females and smokers had a higher risk of contracting age-related cataracts than males and never-smokers respectively (Hennis et al., 2004; McCarty et al., 1999; DeBlack et al., 2003). It is, however, important to note that recruited cases were mostly female (142 versus 58 males). Since recruitment was random, the high number of females might indicate a higher prevalence of the disease among Hermel females. Even though dark-colored irises put the subjects at higher risk of contracting age-related cataracts (Hammond et al., 2000), less cases had dark-colored irises than controls. On the other hand, more cases than controls have worked in agriculture, and have thus had higher exposure to the sun; in the literature, UV light exposure has been consistently found to be associated with age-related cataracts (McCarty & Taylor, 2002).

This study demonstrates, in agreement with the literature, that intakes of lutein and zeaxanthin, β-carotene, vitamin A and vitamin C are inversely related to age-related cataracts (Mares-Perlman et al., 1995; Chasan-Taber et al., 1999; Lyle et al., 1999a; Taylor et al., 1991, 2002; Mares-Perlman et al., 2000; Jacques et al., 2001; Chylack et al., 2002). Intake of α-tocopherol might possibly be related as well to age-related cataracts (Vitale et al., 1993; Leske et al., 1998; Rouhiainen et al., 1996; Mares-Perlman et al., 2000); however, the average intake values reported in this study for α-tocopherol were extremely low, most likely because the semi-quantitative food frequency questionnaire that was used did not include vegetable oil and nuts, which are the richest sources of α-tocopherol along with olive oil in the study population's diet. In Lebanon, olive oil is most frequently consumed raw and rarely used in cooking because it is generally more

expensive; the most common cooking fat is vegetable oil (Nasreddine et al., 2006). Around 18% of the study participants had a low intake of olive oil compared to the average intake of olive oil per capita per day in Lebanon (FAOSTAT, 2003), probably due to under-reporting, which partly explains the low energy intake .

In fact, even though the food frequency questionnaire was not exhaustive, considerable under-reporting of energy intake is likely to have occurred as the 90^{th} percentiles values for all study participants were below the FAO/WHO/UNU recommended average energy intake values. A number of factors are associated with low-energy reporting including weight status, age, sex effects, socioeconomic effects, health-related activities, behavioral effects, and psychological effects (Livingstone & Black, 2003). However, the single most consistent factor related to under-reporting is weight status: the probability that a subject will under-report generally increases as BMI increases (Briefel et al., 1997; Johansson et al., 1998) and as previously stated, 81% of the study participants had a BMI over 25 kg/m^2. The Goldberg cut-off method, which evaluates self-reported energy intakes against estimated energy requirements, could not be applied since food frequency questionnaires are designed to represent a person's usual eating habits over a period of time and are not a precise measure of energy intake. A 24-hour recall could have probably better described the diet in terms of energy intake (Gibson, 2005). Consequently, the quantiles of intake of lutein and zeaxanthin, β-carotene, vitamin A, vitamin C, and α-tocopherol are probably inaccurate; therefore the comparison among cases and controls is of interest rather than the absolute values themselves which need to be interpreted more cautiously.

Even though the average daily intake of lutein and zeaxanthin was significantly correlated with the intake of wild leafy greens ($\rho = 0.332$, $p<0.01$), the major contributors of lutein and zeaxanthin in the diet of both cases and controls did not include wild leafy greens. This could be either due to high dietary

diversity in both groups (see later) or to incorrect nutrient content information for wild leafy greens (as data were not available for most wild leafy greens and average nutrient content values had to be estimated from databases).

Anecdotal evidence indicates that there is no taboo against the consumption of wild leafy greens. On the contrary, most people would consume wild leafy greens if the latter were readily available to them. As a matter of fact, over 90% of both cases and controls consumed wild leafy greens while only 40% collected them. In this regard, it is noteworthy to mention that our study participants were relatively old (average age is 66) and most likely relied on their offspring to supply them with wild leafy greens since it is common practice in the area where the study took place to collect wild edible plants in the nearby agricultural fields, gardens, and mountains, during their growth season.

Results showed that there was no difference in intake of wild leafy greens between cases and controls (although the variability in intake was very high). When the number of wild leafy greens consumed over the last three months was divided by the total number of food items consumed, a significant difference was observed between participants who classified themselves as "poor" and others as "middle-class" (Mann-Whitney U test, $P<0.04$). In other words, participants who classified themselves as "poor" consumed more wild leafy greens than their "middle-class" counterparts regardless of whether they had age-related cataracts or not. Therefore, wild leafy greens are substantially contributing to the quality of the diet (of those who classify themselves as "poor") through improving the micronutrient content of the latter (Humphry et al., 1993; Ogle et al., 2001). In fact, knowledge about wild plants, especially in poor rural areas, is often associated with better health status. In a study by McDade et al. (2007) conducted in the Bolivian Amazon, mothers with higher levels of plant knowledge and use had healthier children, independent of potentially confounding variables related to education, market participation, and acculturation.

Approximately 90% of those who collected wild leafy greens learned about their identification and collection from their parents and that was one of the strongest predictors of wild leafy greens' intake. Cultural transmission from parent-to-child is called vertical, and vertical transmission is associated with slower change in knowledge systems and is thus protective of traditional knowledge (Cavalli-Sforza & Feldman, 1981). Vertical transmission also helps to maintain diversity of knowledge and beliefs within a population (Hewlett & Cavalli-Sforza, 1986). Therefore, traditional knowledge is probably still strong in the community where the study took place, and is ensuring continued use of wild plants.

Other predictors of wild leafy greens' intake included agriculture (as past or present occupation) and unemployment. Both of these predictors are indicative of poor socio-economic status. In this study, participants were asked to rate their subjective perception of their socio-economic status and more than two-thirds of them classified themselves as poor. Asset-based assessments of socio-economic status, such as household survey and participatory wealth ranking (Hargreaves et al., 2007), could not be done because of time and financial constraints.

Nonetheless, the R-squared value of the derived model was of only 0.173, meaning that the above-mentioned variables were not exclusively able to predict the intake of wild leafy greens. Other variables, such as access for example (Swindale & Bilinsky, 2005), as well as random effects related to intake may partially account for such a low R-squared value. However, ethnicity and religion are unlikely to predict intake because, as stated before, the study was conducted in a homogeneous community.

In general, controls had a more diverse diet than cases even though they did not have a significantly higher BMI (Mann-Whitney U test, $P>0.5$) - dietary diversity has been associated with BMI in some studies (Benefice et al., 2007) as access to more diverse foods can sometimes lead to diets higher in fats, causing

weight gain and resulting in health problems (Drewnowski & Popkin, 1997). Controls consumed a significantly higher number of food items rich in lutein and zeaxanthin, β-carotene, vitamin C and α-tocopherol than cases did over a period of three months (Mann-Whitney U test, $p<0.001$). However, the number of food items rich in vitamin A consumed did not differ between cases and controls although the p value obtained was very close to being significant (Mann-Whitney U test, $p>0.5$). The majority of these findings agree with the results obtained from comparing intakes of the above-mentioned nutrients among cases and controls, indicating that there is a positive association between dietary diversity and nutrient intake.

As a matter of fact, adequacy of nutrient intake has been positively associated with the number of different foods consumed (Ferguson et al., 1993; Onyango et al., 1998; Bernstein et al., 2002). Results showed that even though the energy intakes were very low compared to recommended intakes (FAO/WHO/UNU, 2001), average nutrient intakes were above the recommended values for all nutrients except β-carotene and α-tocopherol (knowing that participants at the 90^{th} percentile met the recommendations for β-carotene) suggesting a diet of high quality. As mentioned before, the study area is rural and fairly traditional. The diet is composed of traditional Lebanese dishes and personal field observations indicated very rare use of canned or processed products.

On the other hand, no significant difference was observed between the dietary diversity scores (DDS) of cases and controls neither when leafy greens (Mann-Whitney U test, $p>0.6$) nor when wild leafy greens (Mann-Whitney U test, $p>0.06$) were considered part of the food groups into which the food items were divided. This is probably due to the fact that all groups contained at least one staple food and that the DDS covered a period of three months. Food groups could not be based on nutrient content as most food items (apart from staples) contained two or more of the nutrients studied. The reason why the dietary

diversity scores (DDS) were not significantly different between cases and controls but the food variety scores (FVS) were, could also be explained by a synergistic interaction between lutein and zeaxanthin, β-carotene, vitamin C, α-tocopherol and/or some other phytochemical factor or factors that are associated with the diversity of intake of plant foods.

STUDY VALIDITY

The most serious weaknesses of these findings are potential bias (including selection, information, and confounding bias), and the dietary assessment methods used. Potential selection bias was reduced as most of both cases and controls were selected by random walk. The chance that any control participant might have any form of age-related cataract was minimized by the fact that all controls were tested negative for any eye disease during the last eye check-up that they had. All potential participants approached agreed to take part in the study.

Obviously, it was not possible to blind the interviewers to each participant's disease status. Moreover, participants could not be expected to provide themselves written answers to the questionnaires as more than two-thirds were illiterate and around 30% of cases had not had cataract surgery (and thus had poor visual acuity). Therefore, the interviewers read out all questions clearly, without giving any additional explanations, in order to keep the reliability of the answers to the questions as optimal as possible. There is no reason to suspect that the responses given differed depending on the interviewer as the nutritionist participated in most of the interviews.

Data were collected on the most important potential confounding factors: age, gender, iris color, cigarette smoking and sunlight exposure. These confounders, with their strong interrelations with age-related cataracts, could have had a direct effect on the results obtained, especially that they could have affected

the relationship between the intakes of lutein and zeaxanthin as well as other antioxidants and age-related cataracts. Personal field observations revealed that most of the cases had experienced the death of someone close to them, and thus unusually intense and disruptive feelings of grief, before developing age-related cataracts. Hence, cataracts could have been the result of an increased oxidative stress. Further inquiry into the topic was not possible as war broke out in Lebanon a couple of weeks after the fieldwork was completed.

The semi-quantitative food frequency questionnaire used was modified (from a previously validated food frequency questionnaire used by Dr. Malek Batal's research team to assess food intake -including that of wild plants- in the same area) to include along with staples, local foods particularly rich in lutein and zeaxanthin as well as in other carotenoids (such as β-carotene and lycopene). The semi-quantitative food frequency questionnaire is a widely used tool in dietary assessment. However, it is a relatively crude instrument as it depends on people's recollection at a given point in time of foods they take over long periods of time; it doesn't measure accurately what a person actually consumes every day, or how that changes (Van Staveren et al., 1986; Dwyer & Coleman, 1997; Ambrosini et al., 2003), and can overestimate nutrient intake (George et al., 2004). To circumvent the problem of difficulty with portion estimation, food models were used as in previous studies (Moore et al., 1967; Labadarios, 1999). In addition, the interviewers were trained to ensure the accuracy and consistency of the information obtained.

All twelve common and indigenous wild leafy greens consumed were listed in the questionnaire. In addition, study participants were asked to recall if they consumed any other wild leafy greens and, if so, how frequently based on a usual portion size. As mentioned before, wild leafy greens are highly seasonal, and most of them are available only between the months of February and May. The 3-month food frequency questionnaire spread over most of their growing period to capture as much as possible of the yearly intake.

The Food Variety Score (FVS) and the Dietary Diversity Score (DDS), the two measures of dietary diversity used, have both been shown to reflect nutrient adequacy (Ruel, 2002) and dietary quality (Hatløy et al., 1998; Torheim et al., 2004; Savy et al., 2005) in developing countries. If used alone, the FVS can sometimes give a false favourable impression of the quality of the diet as it counts all the food items consumed, regardless whether they came from the same or different food groups. A high DDS, on the other hand, will reflect a consumption of foods from several of the food groups, and is thus indirectly indicative of a diet of high nutritional quality. Both the FVS and the DDS have the advantage of being simple, low-cost, and widely used methods for the assessment of the nutritional quality of diets (Ogle et al., 2001). Measurement periods for FVS and DDS have most commonly ranged from 1 to 3 or 7 days. However, the present study measures FVS and DDS over a period of three months, which makes its comparison to other studies hard and resulted in generalized high DDS.

STUDY LIMITATIONS

Some limitations of our study must be acknowledged. First, it was impossible to sort out the contribution of lutein and zeaxanthin from that of β-carotene, vitamin C, α-tocopherol, vitamin A and from plant diversity itself. Moreover, the study did not measure the lens optical density of the cases, or determine the subtype of cataracts that the participants had, which would have drawn a stronger relationship between antioxidant intake and age-related cataracts. In addition, intakes of lutein and zeaxanthin, β-carotene, and vitamin A (the different carotenoids studied) were derived from the consumed food items' nutrient composition whereas previous research indicates large variations in bioavailability of carotenoids from different plant foods (DePee et al., 1995; Van het Hof et al., 1999b). The nutrient content of the majority of wild leafy greens was estimated using average values from databases (USDA National Cancer

Institute Carotenoid Food Composition Database; Calvo, 2005; O'Neill et al., 2001) and may not be accurate. The consumption of wild leafy greens depended not only on the seasonality of supply but also on the accessibility and the availability of these plants on the market (which is often impeded by their short shelf-life). Finally, the study results could not be generalized to the heterogeneous Lebanese population which is distributed between very different urban and rural settings.

6.0 CONCLUSION

Age-related eye diseases can be devastating in terms of personal quality of life, as well as national public health and economics. In Lebanon, age-related cataracts are responsible for more than 40% of the causes of blindness (Mansour et al., 1997). The present findings suggest that dietary antioxidants such as lutein and zeaxanthin, β-carotene, vitamin A and vitamin C may contribute to protection against age-related cataracts. Dietary diversity seems to contribute to protection against age-related cataracts by optimizing nutrient intake. In addition, wild leafy greens seem to substantially contribute to the quality of the diet, especially of those of low socio-economic status.

In summary, our findings support recommendations to consume a varied diet rich in fruits and vegetables in order to decrease micronutrient deficiencies and health problems that result from a high reliance on refined foods. They also strongly encourage the conservation of traditional knowledge on the identification, collection and consumption of wild leafy greens.

To our knowledge, the present study is the first to examine the relationship between antioxidant intake and age-related cataracts in a developing country. It is also the first study to look at the importance of traditional wild leafy greens in preventing age-related cataracts. Its main strengths are that it was conducted in an ethnically homogeneous population and that it correlated long-term dietary intake and age-related cataracts.

Cataract research is still a fertile field for investigation as few diseases have as great an impact on public health worldwide. Future studies should better elucidate the relation between antioxidant intake and specific opacity subtypes. Relative risk of age-related cataracts in relation to intake of antioxidants could help sort out the contribution of lutein and zeaxanthin from that of other antioxidants after adjusting for confounding variables (Snellen, 2002). The

relationship between age-related cataracts and wild leafy greens should be investigated further, particularly in populations that are food insecure. In addition, prospective public health interventions should not undermine the use of wild leafy greens, but should, on the opposite, bolster it and study ways to prolong the period of availability of these plants as they seem to constitute the major source of key micronutrients, especially in the rural areas of developing countries (Humphry et al., 1993; Ogle et al., 2001; Batal & Hunter, 2007) where the rates of cataract are the highest (Xu et al., 2006; Chandrashekhar et al., 2007; Mathenge et al., 2007).

7.0 REFERENCES

- **Age-Related Eye Disease Study Research Group**. (2001a). A randomized placebo-controlled clinical trial of high-dose supplementation with vitamins C and E, beta-carotene, and zinc for age-related macular degeneration and vision loss. AREDS report no.8. *Archives of Ophthalmology, 119,* 1147-1436.

- **Age-Related Eye Disease Study Research Group**. (2001b). A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E and beta carotene for age-related cataract and vision loss: AREDS report no. 9. *Archives of Ophthalmology, 119*, 1439–1452.

- **Alberti, G., Oguni, M., Podgor, M., Sperduto, R.D., Tomarev, S., Grassi, C., et al.** (1996). Glutathione S-transferase M1 genotype and age-related cataracts: lack of association in an Italian population. *Investigative Ophthalmology & Visual Science, 37,* 1167-1173.

- **Alves-Rodrigues, A., & Shao, A**. (2004). The science behind lutein. *Toxicology Letters, 150*, 57-83.

- **Amar, I., Aserin, A., & Garti, N**. (2003). Solubilization patterns of lutein and lutein esters in food grade nonionic microemulsions. *Journal of Agriculture and Food Chemistry, 51,* 4775–4781.

- **Ambrosini, G.L., Van Roosbroeck, S.A.H., Mackerras, D., Fritschi, L., de Klerk, N.H., & Musk, A.W.** (2003). The reliability of ten-year dietary recall: implications for cancer research. *Journal of Nutrition, 133,* 2663-2668.

- **Ascherio, A., Rimm, E.B., Hernan, M.A., Giovannucci, E., Kawachi, I., Stampfer, M.J., et al.** (1999). Relation of consumption of vitamin E, vitamin C, and carotenoids to risk for stroke among men in the United States. *Annals of Internal Medicine, 130*, 963-970.

- **Batal, M., & Hunter, E.** (2007). Traditional Lebanese recipes based on wild plants: an answer to diet simplification? *Food and Nutrition Bulletin, 28,* S303-S311.

- **Bates, C.J., Chen, S.J., Macdonald, A., & Holden, R.** (1996). Quantitation of vitamin E and a carotenoid pigment in cataractous human lenses, and the effect of a dietary supplement. *International Journal for Vitamin and Nutrition Research, 66,* 316-321.

- **Beatty, S., Boulton, M., Henson, D., Kob, H.H., & Murray, I.J**. (1999). Macular pigment and age related macular degeneration. *British Journal of Ophthalmology, 83*, 867–877.

- **Beatty, S., Koh, H., Phil, M., Henson, D., & Boulton, M**. (2000). The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Survey of Ophthalmology, 45*, 115-134.

- **Belda, J.I., Romá, J., Vilela, C., Puertas, F.J., Díaz-Llopis, M., Bosch-Morell, F., et al.** (1999). Serum vitamin E levels negatively correlate with the severity of age-related macular degeneration. *Mechanisms of Ageing and Development, 107,* 159-164.

- **Benefice, E., Lopez, R., Monroy, S.L., & Rodriguez, S.** (2007). Fatness and overweight in women and children from riverine Amerindian communities of the Beni River (Bolivian Amazon). *American Journal of Human Biology, 19*, 61-73.

- **Berendschot, T.T., Broekmans, W.M., Klopping-Ketelaars, I.A., Kardinaal, A.F., Van Poppel, G., & Van Norren, D.** (2002). Lens aging in relation to nutritional determinants and possible risk factors for age-related cataract. *Archives of Ophthalmology, 120,* 1732-1737.

- **Bernstein, P.S., Khachik, F., Carvalho, L.S., Muir, G.J., Zhao, D.Y., & Katz, N.B.** (2001). Identification and quantitation of carotenoids and their metabolites in the tissues of the human eye. *Experimental Eye Research, 72,* 215-223.

- **Bernstein, M.A., Tucker, K.L., Ryan, N.D., O'Neill, E.F., Clements, K.M., Nelson, M.E., et al.** (2002). Higher dietary variety is associated with better nutritional status in frail elderly people. *Journal of the American Dietetic Association, 102,* 1096-1104.

- **Bhuyan, K.C., & Bhuyan, D.K.** (1984). Molecular mechanism of cataractogenesis: III. Toxic metabolites of oxygen as initiators of lipid peroxidation and cataract. *Current Eye Research, 3*, 67-81.

- **Bidoli, E., Bosetti, C., La Vecchia, C., Levi, F., Parpinel, M., Talamini, R., et al.** (2003). Micronutrients and laryngeal cancer risk in Italy and Switzerland: a case-control study. *Cancer Causes Control, 14*, 477-484.

- **Beiefel, R.R., Sempos, C.T., McDowell, M.A., Chien, S., & Alaimo, K.** (1997). Dietary method research in the third National Health and Nutrition Examination Survey: Underreporting of energy intake. *American Journal of Clinical Nutrition, 65*, S1203-S1209.

- **Bone, R.A., Landrum, J.T., Fernandez, L., & Tarsis, S.L.** (1988). Analysis of the macular pigment by HPLC: retinal distribution and age study. *Investigative Ophthalmology and Visual Science, 29*, 843-849.

- **Bone, R.A., Landrum, J.T., Friedes, L.M., Gomez, C.M., Kilburn, M.D., Menendez, E., et al.** (1997). Distribution of lutein and zeaxanthin stereoisomers in the human retina. *Experimental Eye Research, 64,* 211-218.

- **Bone, R.A., Landrum, J.T., Hime, G.W., Cains, A., & Zamor, J**. (1993). Stereochemistry of the human macular carotenoids. *Investigative Ophthalmology and Visual Science, 34*, 2033-2040.

- **Bone, R.A., Landrum, J.T., Mayne, S.T., Gomez, C.M., Tibor, S.E., Twaroska, E.E**. (2001). Macular pigment in donor eyes with and without AMD: a case-control study. *Investigative Ophthalmology and Visual Science, 42*, 235-240.

- **Bone, R.A., Landrum, J.T., & Tarsis, S.L**. (1985). Preliminary identification of the human macular pigment. *Vision Research, 25*, 1531-1535.

- **Broekmans, W.M., Klopping-Ketelaars, I.A., Weststrate, J.A., Tijburg, L.B., Van Poppel, G., Vink, A.A., et al.** (2003). Decreased carotenoid concentrations due to dietary sucrose polyesters do not affect possible markers of disease risk in humans. *Journal of Nutrition, 133*, 720-726.

- **Bron, A.J., Vrensen, G.F., Koretz, J., Maraini, G., & Harding, J.J**. (2000). The ageing lens. *Ophthalmologica, 214*, 86-104.

- **Brown, L., Rimm, E.B., Seddon, J.M., Giovannucci, E.L., Chasan-Taber, L., Spiegelman, D., et al.** (1999). A prospective study of carotenoid intake and risk of cataract extraction in US men. *American Journal of Clinical Nutrition, 70*, 517–524.

- **Burke, J.D., Curran-Celentano, J., & Wenzel, A.J.** (2005). Diet and serum carotenoid concentrations affect macular pigment optical density in adults 45 years and older. *Journal of Nutrition, 135*, 1208-1214.

- **Calloway, D.H., Giauque, R.D., & Costa, F.M**. (1974). The superior mineral content of some American Indian foods in comparison to federally donated counterpart commodities. *Ecology of Food and Nutrition, 3*, 203-211.

- **Calvo, M.M**. (2005). Lutein: a valuable ingredient of fruit and vegetables. *Critical Reviews in Food Science and Nutrition, 45*, 671-696.

- **Carnahan, M.C., & Goldstein, D.A.** (2000). Ocular complications of topical, peri-ocular, and systemic corticosteroids. *Current Opinion in Ophthalmology, 11,* 478-483.

- **Castenmiller, J.J.M., West, C. E., Linssen, J.P.H., Van het Hof, K. H., & Voragen, A.G.J**. (1999). The food matrix of spinach is a limiting factor in determining the bioavailability of ß-carotene and to a lesser extent of lutein in humans. *Journal of Nutrition, 129*, 349-355.

- **Cavalli-Sforza, L.L., & Feldman, M.W.** (1981). *Cultural transmission and evolution: A quantitative approach*. Princeton, N.J.: Princeton University Press.

- **Chandrashekhar, T.S., Bhat, H.V., Pai, R.P., & Nair, S.K.** (2007). Prevalence of blindness and its causes among those aged 50 years and above in rural Karnataka, South India. *Tropical Doctor, 37*, 18-21.

- **Chasan-Taber, L., Willett, W.C., Seddon, J.M., Stampfer, M.J., Rosner, B., Colditz, G.A., et al.** (1999). A prospective study of

carotenoid and vitamin A intakes and risk of cataract extraction in US women. *American Journal of Clinical Nutrition, 70*, 509–516.

- **Chitchumroonchokchai, C., Bomser, J.A., Glamm, J.E., & Failla, M.L.** (2004). Xanthophylls and alpha-tocopherol decrease UVB-induced lipid peroxidation and stress signaling in human lens epithelial cells. *Journal of Nutrition, 134,* 3225-3232.

- **Christen, W.G., Ajani, U.A., Glynn, R.J., Manson, J.E., Schaumberg, D.A., Chew, E.C., et al.** (1999). Prospective cohort study of vitamin supplement use and the risk of age-related maculopathy. *American Journal of Epidemiology, 149,* 476-484.

- **Christen, W.G., Manson, J.E., Glynn, R.J., Gaziano, J.M., Sperduto, R.D., Buring, J.E., et al.** (2003). A randomized trial of beta carotene and age-related cataract in US physicians. *Archives of Ophthalmology, 121,* 372-378.

- **Chumlea, W.C., Guo, S.S., & Steinbaugh, M.L.** (1994). Prediction of stature from knee height for black and white adults and children with application to mobility-impaired or handicapped persons. *Journal of the American Dietetic Association, 94,* 1385-1388.

- **Chylack Jr., L.T., Brown, N.P., Bron, A., Hurst, M., Köpcke, W., Thien, U., et al.** (2002). The Roche European American Cataract Trial (REACT): a randomized clinical trial to investigate the efficacy of an oral antioxidant micronutrient mixture to slow progression of age-related cataract. *Ophthalmic Epidemiology, 9*, 49–80.

- **Clevidence, B.A., and Bieri, J.G.** (1993). Association of carotenoids with human plasma lipoproteins. *Methods in Enzymology, 214*, 33-46.

- **Coren, S., & Girgus, J.S.** (1972). Density of human lens pigmentation, in vivo measures over an extended age range. *Vision Research, 12,* 343-346.

- **Darmon, N.**, & **Khlat, M.** (2001). An overview of the health status of migrants in France, in relation to their dietary practices. *Public Health Nutrition, 4,* 163-172.

- **DeBlack, S.S.** (2003). Cigarette smoking as a risk factor for cataract and age-related macular degeneration: a review of the literature. *Optometry, 74,* 99-110.

- **Delcourt, C., Cristol, J.P., Tessier, F., Leger, C., Descomps, B., & Papoz, L.** (1999). The POLA Study Group. Age-related macular degeneration and antioxidant status in the POLA study. *Archives of Ophthalmology, 117,* 1384-1390.

- **DePee, S., West, C.E., Muhilal, Kayardi, D., & Hautvast, J.G.** (1995). Lack of improvement of vitamin A status with increased consumption of dark-green leafy vegetables. *Lancet, 346*, 75-81.

- **Dorey, C.K., Granata, L., Nichols, C.R., Cheng, K.M., & Craft, N.E.** (2005). Dietary modulation of lens zeaxanthin in quail. *Experimental Eye Research, 81,* 464–477.

- **Dorey, K., Toyoda, Y., Thomsom, L., Garnett, K. M., Sapunzakis, M., Craft, N., et al.** (1997). Light-induced photoreceptor apoptosis is correlated with dietary and retinal levels of 3R,3'R-zeaxanthin. *Investigative Ophthalmology and Visual Science, 38*, S355.

- **Drewnowski, A., & Popkin, B.M.** (1997). The nutrition transition: New trends in the global diet. *Nutrition Reviews, 55*, 31-43.

- **Dwyer, J.T., & Coleman, K.A.** (1997). Insights into dietary recall from a longitudinal study: accuracy over four decades. *American Journal of Clinical Nutrition, 65*, S1153-S1158.

- **Dwyer, J.H., Navab, M., Dwyer, K.M., Hassan, K., Sun, P., Shircore, A., et al.** (2001). Oxygenated carotenoid lutein and progression of early atherosclerosis: the Los Angeles atherosclerosis study. *Circulation, 103*, 2922-2927.

- **The Eye Disease Case Control Study Group.** (1992). Risk factors for neovascular age-related macular degeneration. *Archives of Ophthalmology, 110,* 1701-1708.

- **The Eye Disease Case-Control Study Group**. (1993). Antioxidant status and neovascular age-related macular degeneration. *Archives of Ophthalmology, 111*, 104–109.

- **Falsini, B., Piccardi, M., Iarossi, G., Fadda, A., Merendino, E., & Valentini, P**. (2003). Influence of short term antioxidant supplementation on macular function in age-related maculopathy: a pilot study including electrophysiologic assessment. *Ophthalmology, 110,* 51–60.

- **FAO/WHO**. (2002). *Human vitamin and mineral requirements. Report of a joint FAO/WHO expert consultation*. Rome: FAO.

- **FAO/WHO/UNU**. (2001). *Human energy requirements. Report of a joint FAO/WHO/UNU expert consultation*. Rome: FAO.

- **Farhat, C.** (2006). *Analysis of indigenous nutritional knowledge, cultural importance and nutritional analysis of wild edible plants*. Unpublished master's thesis. American University of Beirut, Beirut.

- **Ferguson, E.L., Gibson, R.S., Opare-Obisaw, C., Osei-Opare, F., Lamba, C., & Ounpuu, S.** (1993). Seasonal food consumption patterns and dietary diversity of rural preschool Ghanaian and Malawian children. *Ecology of Food and Nutrition, 49*, 565-578.

- **Fite, K.V., & Bengston, L.** (1989). Aging and sex-related changes in the outer retina of Japanese quail. *Current Eye Research, 8,* 1039-1048.

- **Flood, V., Smith, W., Wang, J.J., Manzi, F., Webb, K., & Mitchell, P.** (2002). Dietary antioxidant intake and incidence of early age-related maculopathy. *Ophthalmology, 109,* 2272-2278.

- **Gale, C.R., Hall, N.F., Phillips, D.I., & Martyn, C.N.** (2001). Plasma antioxidant vitamins and carotenoids and age-related cataract. *Ophthalmology, 108,* 1992-1998.

- **George, G.C., Milani, T.J., Hanss-Nuss, H., Kim, M., & Freeland-Graves, J.H.** (2004). Development and validation of a semi-quantitative food frequency questionnaire for young adult women in the southwestern United States. *Nutrition Research, 24,* 29-43.

- **Gibson, R.S.** (2005). *Principles of Nutritional Assessment.* New York: Oxford University Press.

- **Glynn, R.J., Christen, W.G., Manson, J.E., Bernheimer, J., & Hennekens, C.H.** (1995). Body mass index: an independent predictor of cataract. Archives of *Ophthalmology, 113,* 1131-1137.

- **Goldberg, J., Flowerdew, G., Smith, E., Brody, J.A., & Tso, M.O.M.** (1988). Factors associated with age-related macular degeneration. *American Journal of Epidemiology, 128,* 700-710.

- **Grivetti, L.E., & Ogle, B.M**. (2000). Value of traditional foods in meeting macro- and micronutrient needs: the wild plant connection. *Nutrition Research Reviews, 13*, 31-46.

- **Haegerstrom-Portnoy, G**. (1988). Short-wavelength-sensitive-cone sensitivity loss with aging: a protective role for macular pigment? *Journal of the Optical Society of America. A, Optics, image science, and vision, 5*, 2140–2144.

- **Hammond, B.R., Nanez, J.E., Fair, C., & Snodderly, D.M.** (2000). Iris color and age-related changes in lens optical density. *Ophthalmic and Physiological Optics, 5,* 381-386.

- **Hammond, B.R., Wooten, B.R., & Curran-celentano, J**. (2001). Carotenoids in the retina and lens: possible acute and chronic effects on human visual performance. *Archives of Biochemistry and Biophysics, 385*, 41-46.

- **Hammond, B.R., Wooten, B.R., & Snodderly, D.M**. (1997).Density of the human crystalline lens is related to the macular pigment carotenoids, lutein and zeaxanthin. *Optometry and Vision Science, 74*, 499–504.

- **Handelman, G.J., Dratz, E.A., Reay, C.C., & Van Kujik, J.G**. (1988). Carotenoids in the human macula and whole retina. *Investigative Ophthalmology and Visual Science, 29*, 850-855.

- **Hankinson, S.E., Stampfer, M.J., Seddon, J.M., Colditz, G.A., Rosner, B.N., Speizer, F.E., et al.** (1992). Nutrient intake and cataract extraction in women: a prospective study. *British Medical Journal, 305*, 335–339.

- **Hargreaves, J.R., Morison, L.A., Gear, J.S., Kim, J.C., Makhubele, M.B., Porter, J.D., et al.** (2007). Assessing household wealth in health studies in developing countries: A comparison of participatory wealth ranking and survey techniques from rural South Africa. *Emerging Themes in Epidemiology, 4*, 1-9.

- **Hatløy, A., Torheim, L.E., & Oshaug, A.** (1998). Food variety – a good indicator of nutritional adequacy of the diet? A case study from an urban area in Mali, West Africa. *European Journal of Clinical Nutrition, 52,* 891-898.

- **Heiba, I.M., Elston, R.C., Klein, B.E., & Klein, R.** (1994). Sibling correlations and segregation analysis of age-related maculopathy: The Beaver Dam Eye Study. *Genetic Epidemiology, 11,* 51-67.

- **Hennis, A., Wu, S.Y., Nemesure, B., & Leske, M.C.** (2004). Risk factors for incident cortical and posterior subcapsular lens opacities in the Barbados Eye Studies. *Archives of Ophthalmology, 122,* 525-530.

- **Hewlett, B., & Cavalli-Sforza, L.L.** (1986). Cultural transmission among Aka pygmies. *American Anthropologist, 88*, 922-934.

- **Heywood, V.H.** (1999). *The Mediterranean Region: A major centre of plant diversity. Centre international de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM)*. Retrieved February 28, 2006, from http://ressources.ciheam.org/om/pdf/c38/CI020525.pdf.

- **Hiller, R., Podgor, M.J., Sperduto, R.D., Nowroozi, L., Wilson, P.W., D'Agostino, R.B., et al.** (1998) A longitudinal study of body mass index and lens opacities. The Framingham Studies. *Ophthalmology, 105,* 1244-1250.

- **Hoddinott, J., & Yohannes, Y. (**2002). *Dietary diversity as a household food security indicator.* Washington, DC: Food and Nutrition Technical Assistance Project, Academy for Educational Development.

- **Holden, J.M, Eldridge, A.L., Beecher, G.R., Buzzard, M.I., Bhagwat, S., Davis, C. S., et al.** (1998). Carotenoid content of US foods: an update of the database. *Journal of Food Composition and Analysis, 12,* 169–196.

- **Humphry, C.M., Clegg, M.S., Keen, C.L., & Grivetti, L.E**. (1993). Food diversity and drought survival. The Hausa example. *International Journal of Food Sciences and Nutrition, 44*, 1-16.

- **Hyman, L., Schachat, A.P., He, Q., & Leske, M.C.** (2000). Hypertension, cardiovascular disease, and age-related macular degeneration. Age-Related Macular Degeneration Risk Factors Study Group. *Archives of Ophthalmology, 118,* 351-358.

- **Institute of Food Technologists.** (1975). Naturally occurring toxicants in foods. A scientific status summary by the institute of food technologists' expert panel on food safety and nutrition. *Journal of Food Science, 40,* 215–222.

- **Iwig, M., Glaesser, D., Fass, U., & Struck, H.G.** (2004). Fatty acid cytotoxicity to human lens epithelial cells. *Experimental Eye Research, 79,* 689-704.

- **Jacques, P.F., Chylack Jr., L.T., Hankinson, S.E., Khu, P.M., Rogers, G., Friend, J., et al.** (2001). Long-term nutrient intake and early age-related nuclear lens opacities. *Archives of Ophthalmology, 119,* 1009-1019.

- **Jacques, P.F., Moeller, S.M., Hankinson, S.E., Chylack, L.T., Rogers, G., Tung, W.H., et al.** (2003). Weight status, abdominal adiposity, diabetes, and early age-related lens opacities. *American Journal of Clinical Nutrition, 78,* 400-405.

- **James, W.P., Duthie, G.G., & Wahle, K.W.** (1989). The Mediterranean diet: protective or simply non-toxic? *European Journal of Clinical Nutrition, 43,* S31- S41.

- **Johansson, L., Solvoll, K., Bjørneboe, G.E., & Drevon, C.A.** (1998). Under- and overreporting of energy intake related to weight status and lifestyle in a nationwide sample. *American Journal of Clinical Nutrition, 68*, 266-274.

- **Johns, T.** (2003). Plant biodiversity and malnutrition: simple solutions to complex problems. *African Journal of Food, Agriculture, Nutrition and Development, 3,* 45-52.

- **Johnson, E.J**. (2002). The role of carotenoids in human health. *Nutrition in Clinical Care, 5*, 56-65.

- **Johnson, E.J., Hammond, B.R., Yeum, K.J., Qin, J., Wang, X.D., Castaneda, C., et al.** (2000). Relation among serum and tissue concentrations of lutein and zeaxanthin and macular pigment density. *American Journal of Clinical Nutrition, 71,* 1555-1562.

- **Kabuye, C.S., & Ngugi, G.W**. (2001). Nutritional and medicinal importance of indigenous food plants. In *The Potential of Indigenous Wild Foods. Workshop Proceedings*.

- **Kaiser, H.J., Flammer, J., Stumpfig, D., & Hendrickson, P**. (1995). Visaline in the treatment of age-related macular degeneration - A pilot study. *Ophthalmologica, 209,* 302-305.

- **Karrer, P., Salomon, H., & Wehrli, H**. (1929). Pflanzenfarbstoffe XIV. Über einen carotinoidfarbstoff aus mais: Zeaxanthin. *Helvetica Chimica Acta, 12,* 790-792.

- **Kennedy, G., Nantel, G., & Shetty, P.** (2003). The scourge of "hidden hunger": global dimensions of micronutrient deficiencies. *Food, Nutrition and Agriculture, 32,* 8-16.

- **Keys, A.** (1980). *Seven Countries: A Multivariate Analysis of Death and Coronary Heart Disease*. Cambridge, MA: Harvard University Press.

- **Khachik, F., Bernstein, P.S., & Garland, D.L.** (1997). Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Investigative Ophthalmology and Visual Science, 38*, 1802-1811.

- **Khan, J.C., Shahid, H., Thurlby, D.A., Bradley, M., Clayton, D.G., Moore, A.T., et al.** (2006). Age related macular degeneration and sun exposure, iris colour, and skin sensitivity to sunlight. *British Journal of Ophthalmology, 90,* 29-32.

- **Klein, B.E., Klein, R., Jensen, S.C., & Ritter, L.L.** (1994). Are sex hormones associated with age-related maculopathy in women? The Beaver Dam Eye Study. *Transactions of the American Ophthalmological Society, 92,* 289-297.

- **Klein, B.E., Klein, R., & Lee, K.E.** (1998). Diabetes, cardiovascular disease, selected cardiovascular disease risk factors, and the 5-year

incidence of age-related cataract and progression of lens opacities: The Beaver Dam Eye Study. *American Journal of Ophthalmology, 126,* 782-790.

- **Klein, B.E., Klein, R., Lee, K.E., & Jensen, S.C.** (2001a) Measures of obesity and age-related eye diseases. *Ophthalmic Epidemiology, 8,* 251-262.

- **Klein, B.E., Klein, R., Lee, K.E., Moore, E.L., & Danforth, L.** (2001b). Risk of incident age-related eye diseases in people with an affected sibling: The Beaver Dam Eye study. *American Journal of Epidemiology, 154,* 207-211.

- **Klein, B.E., Klein, R., & Linton, K.L.** (1992). Prevalence of age-related lens opacities in a population: The Beaver Dam Eye Study. *Ophthalmology, 99,* 546-552.

- **Klein, R., Klein, B.E.K., & Franke, T.** (1993). The relationship of cardiovascular disease and its risk factors to age-related maculopathy. The Beaver Dam Eye Study. *Ophthalmology, 100,* 406-414.

- **Klein, R., Klein, B.E., Tomany, S.C., & Cruickshanks, K.J.** (2003a). The association of cardiovascular disease with the long-term incidence of age-related maculopathy: The Beaver Dam Eye study. *Ophthalmology, 110,* 1273-1280.

- **Klein, R., Klein, B.E., Tomany, S.C., Danforth, L.G., & Cruickshanks, K.J.** (2003b). Relation of statin use to the 5-year incidence and progression of age-related maculopathy. *Archives of Ophthalmology, 121,* 1151-1155.

- **Krinsky, N.I., & Johnson, E.J**. (2005).Carotenoid actions and their relation to health and disease. *Molecular Aspects of Medicine, 26*, 459-516.

- **Kuczmarski, K.F., Kuczmarski, R.J., & Najjar, M.** (2000).Descriptive anthropometric reference data for older Americans. *Journal of the American Dietetic Association, 100*, 59-66.

- **Kuhnlein, H.V., & Receveur, O**. (1996). Dietary change and traditional food systems of Indigenous Peoples. *Annual Reviews in Nutrition, 16*, 417-442.

- **Labadarios, D.** (1999). *The National Food Consumption Survey (NFCS): Children aged 1-9 years, South Africa*. Pretoria: Department of Health.

- **Landrum, J.T., & Bone, R.A**. (2001). Lutein, zeaxanthin, and the macular pigment. *Archives of Biochemistry and Biophysics, 385*, 28-40.

- **Landrum, J.T., Bone, R.A., & Kilburn, M.D**. (1997). The macular pigment: a possible role in protection from age-related macular degeneration. *Advances in Pharmacology, 38*, 537-556.

- **Leske, M.C., Chylack Jr., L.T., He, Q., Wu, S.Y., Schoenfeld, E., Friend, J., et al.** (1998). Antioxidant vitamins and nuclear opacities: the longitudinal study of cataract. *Ophthalmology, 105,* 831-836.

- **Livingstone, M.B.E, & Black, A.E.** (2003). Markers of the validity of reported energy intake. *Journal of Nutrition, 133,* S895-S920.

- **Lonely Planet**. *Map of Lebanon*. Retrieved February 28, 2006, from http://www.lonelyplanet.com/mapshells/middle_east/lebanon/lebanon.htm .

- **Lu, M., Cho, E., Taylor, A., Hankinson, S.E., Willett, W.C., & Jacques, P.F.** (2005). Prospective study of dietary fat and risk of cataract extraction among US women. *American Journal of Epidemiology, 161,* 948-959.

- **Lyle, B.J., Mares-Perlman, J.A., Klein, B.E.K., Klein, R., & Greger, J.L**. (1999a). Antioxidant intake and risk of incident age-related nuclear cataracts in the Beaver Dam Eye Study. *American Journal of Epidemiology, 149,* 801–809.

- **Lyle, B.J., Mares-Perlman, J.A., Klein, B.E.K., Klein, R., Palta, M., Bowen, P.E., et al.** (1999b). Serum carotenoids and tocopherols and incidence of age-related nuclear cataract. *American Journal of Clinical Nutrition, 69*, 272–277.

- **The Macular Degeneration Partnership.** (2007). *Types of AMD*. Retrieved July 21, 2007, from http://www.amd.org/site/ PageServer?pagename=Types_of_AMD.

- **Malek, G., Li, C.M., Guidry, C., Medeiros, N.E., & Curcio, C.A.** (2003). Apolipoprotein B in cholesterol-containing drusen and basal deposits of human eyes with age-related maculopathy. *American Journal of Pathology, 162,* 413-425.

- **Malinow, M.R., Feeney-Burns, L., Peterson, L.H., Klein, M.L., & Neuringer, M**. (1980). Diet-related macular anomalies in monkeys. *Investigative Ophthalmology and Visual Science, 19*, 857-863.

- **Mansour, A.M., Kassak, K., Chaya, M., Hourani, T., Sibai, A., & Alameddine, M.N**. (1997). National survey of blindness and low vision in Lebanon. *British Journal of Ophthalmology, 81*, 905-906.

- **Maraini, G., Hejtmancik, J.F., Shiels, A., Mackay, D.S., Aldigeri, R., Jiao, X.D., et al.** (2003). Galactokinase gene mutations and age-related cataract. Lack of association in an Italian population. *Molecular Vision, 9,* 397-400.

- **Mares-Perlman, J.A., Brady, W.E., Klein, R., Klein, B.E.K., Bowen, P., Stacewicz-Sapuntzakis, M., et al.** (1995a). Serum antioxidants and age-related macular degeneration in a population-based case-control study. *Archives of Ophthalmology, 113,* 1518-1523.

- **Mares-Perlman, J.A., Brady, W.E., Klein, B.E., Klein, R., Palta, M., Bowen, P., et al.** (1995b). Serum carotenoids and tocopherols and severity of nuclear and cortical opacities. *Investigative Ophthalmology and Visual Science, 36*, 276–288.

- **Mares-Perlman, J.A., Fisher, A.I., Klein, R., Palta, M., Block, G., Millen, A.E., et al.** (2001). Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *American Journal of Epidemiology, 153*, 424-432.

- **Mares-Perlman, J.A., Lyle, B.J., Klein, R., Fisher, A.I., Brady, W.E., VandenLangenberg, G.M., et al.** (2000). Vitamin supplement use and incident cataracts in a population-based study. *Archives of Ophthalmology, 118,* 1556-1563.

- **Mares-Perlman, J.A., Millen, A.E., Ficek, T.L., & Hankinson, S.E**. (2002). The body of evidence to support a protective role for lutein and zeaxanthin in delaying chronic disease: Overview. *Journal of Nutrition, 132*, S518-S524.

- **Mathenge, W., Kuper, H., Limburg, H., Polack, S., Onyango, O., Nyaga, G., et al.** (2007). Rapid assessment of avoidable blindness in Nakuru district, Kenya. *Ophthalmology, 114,* 599-605.

- **McCarty, C.A., Mukesh, B.N., Fu, C.L., Mitchell, P., Wang, J.J., & Taylor, H.R.** (2001). Risk factors for age-related maculopathy: the Visual Impairment Project. *Archives of Ophthalmology, 119,* 1455-1462.

- **McCarty, C.A., Mukesh, B.N., Fu, C.L., & Taylor, H.R.** (1999). The epidemiology of cataract in Australia. *American Journal of Ophthalmology, 128,* 446-465.

- **McCarty, C.A., & Taylor, H.R.** (2002). A review of the epidemiologic evidence linking ultraviolet radiation and cataracts. *Developments in Ophthalmology, 35,* 21-31.

- **McDade, T.W., Reyes-García, V., Blackinton, P., Tanner, S., Huanca, T., & Leonard, W.R.** (2007). Ethnobotanical knowledge is associated with indices of child health in the Bolivian Amazon. *Proceedings of the National Academy of Sciences of the United States of America, 104*, 6134-6139.

- **McGwin, G., Owsley, C., Curcio, C.A., & Crain, R.J.** (2003). The association between statin use and age related maculopathy. *British Journal of Ophthalmology, 87,* 1121-1125.

- **McNeil, J.J., Robman, L., Tikellis, G., Sinclair, M.I., McCarty, C.A., & Taylor, H.R.** (2004). Vitamin E supplementation and cataract: randomized controlled trial. *Ophthalmology, 111,* 75-84.

- **Micozzi, M.S., Beecher, G.R., Taylor, P.R., & Khachlik, F**. (1990). Carotenoid analyses of selected raw and cooked foods associated with a lower risk for cancer. *Journal of the National Cancer Institute, 82*, 282-285.

- **Moeller, S.M., Jacques, P.F., & Blumberg, J.B**. (2000). The potential role of dietary xanthophylls in cataract and age-related macular degeneration. *Journal of the American College of Nutrition, 19*, S522-S527.

- **Moore, M.C., Judlin, B.C., & Kennemur, P.M.** (1967). Using graduated food models in taking dietary histories. *Journal of the American Dietetic Association, 51*, 447-450.

- **Mozaffarieh, M., Sacu, S., & Wedrich, A**. (2003). The role of the carotenoids, lutein and zeaxanthin, in protecting against age-related macular degeneration: A review based on controversial evidence. *Nutrition Journal, 2*, 20-28.

- **Mukesh, B.N., Le, A., Dimitrov, P.N., Ahmed, S., Taylor, H.R., & McCarty, C.A.** (2006). Development of cataract and associated risk factors: the Visual Impairment Project. *Archives of Ophthalmology, 124,* 79-85.
- **Nasreddine, L., Hwalla, N., Sibai, A., Hamzé, M., & Parent-Massin, D.** (2006). Food consumption patterns in an adult urban population in Beirut, Lebanon. *Public Health Nutrition, 9*, 194-203.

- **Newsome, D.A., Swartz, M., Leone, N.C., Elston, R.C., & Miller, E.** (1988). Oral zinc in macular degeneration. *Archives of Ophthalmology, 106,* 192-198.

- **Nicolas, C.M., Robman, L.D., Tikellis, G., Dimitrov, P.N., Dowrick, A., Guymer, R.H., et al.** (2003). Iris colour, ethnic origin and progression of age-related macular degeneration. *Clinical and Experimental Ophthalmology, 31,* 465-469.

- **Ogle, B.M., Hung, P.H., & Tuyet, H.T.** (2001). Significance of wild vegetables in micronutrient intakes of women in Vietnam: An analysis of food variety. *Asia Pacific Journal of Clinical Nutrition, 10*, 21-30.

- **Okano, Y., Asada, M., Fujimoto, A., Ohtake, A., Murayama, K., Hsiao, K.J., et al.** (2001). A genetic factor for age-related cataract: identification and characterization of a novel galactokinase variant, "Osaka," in Asians. *American Journal of Human Genetics, 68,* 1036-1042.

- **Olmedilla, B., Granado, F., Blanco, I., & Vaquero, M.** (2003). Lutein, but not alpha-tocopherol, supplementation improves visual function in patients with age-related cataracts: a 2-y double-blind, placebo-controlled pilot study. *Nutrition, 19*, 21–24.

- **Olson, J.A**. (1999). Carotenoids and human health. *Archivos Latinoamericanos de Nutricion, 49*, S7-S11.

- **O'Neill, M.E., Caroll, Y., Corridan, B., Olmedilla, B., Granado, F., Blanco, I., et al.** (2001). A European carotenoid database to assess carotenoid intakes and its use in a five-country comparative study. *British Journal of Nutrition, 85*, 499-507.

- **Onyango, A., Koski, K.G., & Tucker, K.L.** (1998). Food diversity versus breastfeeding choice in determining anthropometric status in rural Kenyan toddlers. *International Epidemiology Association, 27*, 484-489.

- **Pease, P.L., Adams, A.J., & Nuccio, E**. (1987). Optical density of human macular pigment. *Vision Research, 27*, 705–710.

- **Pfander, H**. (1992). Carotenoids: an overview. *Methods in Enzymology, 213,* 3–13.

- **Plat, J., & Mensink, R. P**. (2001) Effects of diets enriched with two different plant stanol ester mixtures on plasma ubiquinol-10 and fat-soluble antioxidant concentrations. *Metabolism, 50*, 520–529.

- **Pokorny, J., Smith, V.C., & Lutze, M.** (1987). Aging of the human lens. *Applied Optics, 26,* 1437-1440.

- **Reading, V.M., & Weale, R.A**. (1974). Macular pigment and chromatic aberration. *Journal of the Optical Society of America, 64*, 231–234.

- **Richer, S., Stiles, W., Statkute, L., Pulido, J., Frankowski, J., Rudy, D., et al.** (2004). Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic ate-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry, 75*, 216–230.

- **Riso, P., Brusamolino, A., Ciappellano, S., & Porrini, M**. (2003). Comparison of lutein bioavailability from vegetables and supplement. *International Journal for Vitamin & Nutrition Research, 73*, 201-205.

- **Rock, C.L., Thornquist, M.D., Neuhouser, M.L., Kristal, A.R., Neumark-Sztainer, D., Cooper, D.A., et al.** (2002). Diet and lifestyle correlates of lutein in the blood and diet. *Journal of Nutrition, 132*, S525-S530.

- **Roodenburg, A.J.C., Leenen, R., Van het Hof, K.H., Weststrate, J.A., & Tijburg, L.B.M.** (2000). Amount of fat in the diet affects bioavailability of lutein esters but not of α-carotene, β-carotene, and vitamin E in humans. *American Journal of Clinical Nutrition, 71,* 1187–1193.

- **Rouhiainen, P., Rouhiainen, H., & Salonen, J.T.** (1996). Association between low plasma vitamin E concentration and progression of early cortical lens opacities. *American Journal of Epidemiology, 144,* 496-500.

- **Ruel, M.T.** (2002). *Is dietary diversity an indicator of food security or dietary quality? A review of measurement issues and research needs.* Washington, D.C.: International Food Policy Research Institute.

- **Sample, P.A., Esterson, F.D., Weinreb, R.N., & Boynton, R.M.** (1988). The aging lens, in vivo assessment of light absorption in 84 human eyes. *Investigative Ophthalmology and Visual Science, 29,* 1306-1311.

- **Sanders, T.A.B., Haines, A.P., Wormald, R., Wright, L.A., & Obeid, O.** (1993). Essential fatty acids, plasma cholesterol, and fat-soluble vitamins in subjects with age-related maculopathy and matched control subjects. *American Journal of Clinical Nutrition, 57*, 428–433.
- **Savy, M., Martin-Prével, Y., Sawadogo, P., Kameli, Y., & Delpeuch, F.** (2005). Use of variety/diversity scores for diet quality measurement: Relation with nutritional status of women in a rural area in Burkina Faso. *European Journal of Clinical Nutrition, 59*, 703-716.

- **Saxena, S., Mitchell, P., & Rochtchina, E.** (2004). Five-year incidence of cataract in older persons with diabetes and pre-diabetes. *Ophthalmic Epidemiology, 11,* 271-277.

- **Schmidt-Erfurth, U.** (2005). Nutrition and retina. *Developments in Ophthalmology, 38*, 120-147.

- **Schröder, H.** (2007). Protective mechanisms of the Mediterranean diet in obesity and type 2 diabetes. *Journal of Nutritional Biochemistry, 18,* 149-160.

- **Seddon, J.M., Ajani, U.A., Sperduto, R.D., Hiller, R., Blair, N., Burton, T.C., et al.** (1994). Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *Journal of the American Medical Association, 272*, 1413–1420.

- **Sekine, Y., Hommura, S., & Harada, S.** (1995). Frequency of glutathione-S-transferase 1 gene deletion and its possible correlation with cataract formation. *Experimental Eye Research, 60,* 159-163.

- **Silvestri, G., Johnston, P.B., & Hughes, A.E.** (1994). Is genetic predisposition an important risk factor in age-related macular degeneration? *Eye, 8,* 564-568.

- **Simopoulos A.P.** (2001). The Mediterranean Diets: What is so special about the diet of Greece? The Scientific Evidence. *Journal of Nutrition, 131,* S3065-S3073.

- **Smith, W., Mitchell, P., & Rochester, C.** (1997). Serum beta carotene, alpha-tocopherol and age-related maculopathy: The Blue Mountains Eye Study. *American Journal of Ophthalmology, 126,* 838-840.

- **Snellen, E.L.M., Verbeek, A.L.M., Van den Hoogen, G.W.P., Cruysberg, J.R.M., & Hoyng, C.B.** (2002). Neovascular age-related macular degeneration and its relationship to antioxidant intake. *Acta Ophthalmologica Scandinavica, 80,* 368-371.

- **Sommerburg, O., Keunen, J.E.E., Bird, A.C., & Van Kiujk, F.J.G.M.** (1998). Fruits and vegetables that are sources for lutein and zeaxanthin: the macular pigment in human eyes. *British Journal of Ophthalmology, 82,* 907–910.

- **Stur, M., Tittl , M., Reitner, A., & Meisinger, V.** (1996). Oral zinc and the second eye in age-related macular degeneration. *Investigative Ophthalmology and Visual Science, 37,* 1225-1235.

- **Swindale, A., & Bilinsky, P.** (2005). *Household dietary diversity score (HDDS) for measurement of household food access: Indicator guide.* Washington, D.C.: Food and Nutrition Technical Assistance Project, Academy for Educational Development.

- **Tabbara, K.F.** (2001). Blindness in the eastern Mediterranean countries. *British Journal of Ophthalmology, 85*, 771-775.

- **Taylor, A., Jacques, P.F., Chylack Jr., L.T., Hankinson, S.E., Khu, P.M., Rogers, G., et al.** (2002). Long-term intake of vitamins and carotenoids and odds of early age-related cortical and posterior subcapsular lens opacities. *American Journal of Clinical Nutrition, 75,* 540-549.

- **Taylor, A., Jacques, P.F., Nadler, D., Morrow, F., Sulsky, S.I., & Shepard, D.** (1991). Relationship in humans between ascorbic acid consumption and levels of total and reduced ascorbic acid in lens, aqueous humor, and plasma. *Current Eye Research, 10,* 751-759.

- **Teikari, J.M., Laatikainen, L., Virtamo, J., Haukka, J., Rautalahti, M., Liesto, K., et al.** (1998). Six-year supplementation with alpha-tocopherol and beta-carotene and age-related maculopathy. *Acta Ophthalmologica Scandinavica, 76,* 224-229.

- **Teikari, J.M., Virtamo, J., Rautalahti, M., Palmgren, J., Liesto, K., & Heinonen, O.P.** (1997). Long-term supplementation with alpha-tocopherol and beta-carotene and age-related cataract. *Acta Ophthalmologica Scandinavica, 75,* 634-640.

- **Thomson, L.R., Toyoda, Y., Langner, A., Delori, F.C., Garnett, K.M., Craft, N., et al.** (2002). Elevated retinal zeaxanthin and prevention of light-induced photoreceptor cell death in quail. *Investigative Ophthalmology and Visual Science, 43,* 3538-3549.

- **Thornton, J., Edwards, R., Mitchell, P., Harrison, R.A., Buchan, I., & Kelly, S.P.** (2005). Smoking and age-related macular degeneration: a review of association. *Eye, 19,* 935-944.

- **Thudichum, J.L.W**. (1869). Researches conducted for the medical department of the Privy Council, at the Pathological Laboratory of St. Thomas's Hospital. Third series. Results of researches on luteine and the spectra of yellow organic substances contained in animals and plants. *Proceedings of the Royal Society of London, 17,* 253–256.

- **Tikellis, G., Robman, L.D., Harper, C.A., Garrett, S.K.M., Mc-Neil, J.J., Taylor, H.R., et al.** (1999). The VECAT study: Methodology and statistical power for measurement of age-related macular features. *Ophthalmic Epidemiology, 6,* 181-194.

- **Toogood, J.H., Markov, A.E., Baskerville, J., & Dyson, C.** (1993). Association of ocular cataracts with inhaled and oral steroid therapy during long-term treatment of asthma. *Journal of Allergy and Clinical Immunology, 91,* 571-579.

- **Trevithick-Sutton, C.C., Foote, C.S., Collins, M., & Trevithick, J.R.** (2006). The retinal carotenoids zeaxanthin and lutein scavenge superoxide and hydroxyl radicals: a chemiluminescence and ESR study. *Molecular Vision, 12,* 1127-1135.

- **Trichopoulou, A.** (2004). Traditional Mediterranean diet and longevity in the elderly: a review. *Public Health Nutrition, 7,* 943-947.

- **Tukan, S.K., Takruri, H.R.M., & Al-Eisawi, D.M**. (1998). The use of wild edible plants in the Jordanian diet. *International Journal of Food Sciences and Nutrition, 49*, 225-235.

- **Tyssandier, V., Cardinault, N., Caris-Veyrat, C., Amiot, M.J., Grolier, P., Bouteloup, C., et al.** (2002). Vegetable-borne lutein, lycopene, and beta-carotene compete for incorporation into chylomicrons, with no adverse effect on the medium-term (3-wk) plasma status of carotenoids in humans. *American Journal of Clinical Nutrition, 75,* 526-534.

- **United States Library of Congress**. *Country Studies: Lebanon*. Retrieved February 28, 2006, from http://countrystudies.us/lebanon/.

- **Van het Hof, K.H., Brouwer, I.A., West, C.E., Haddeman, E., Steegers-Theunissen, R.P., Van Dusseldorp, M., et al.** (1999a). Bioavailability of lutein from vegetables is 5 times higher than that of beta-carotene. *American Journal of Clinical Nutrition, 70,* 261-268.

- **Van het Hof, K.H., Tijburg, L.B., Pietrzik, K., & Weststrate, J.A.** (1999b). Influence of feeding different vegetables on plasma levels of carotenoids, folate and vitamin C. Effect of disruption of the vegetable matrix. *British Journal of Nutrition, 82*, 203-212.

- **Van het Hof, K.H., West, C.E., Weststrate, J.A., & Hautvast, J.G**. (2000). Dietary factors that affect the bioavailability of carotenoids. *Journal of Nutrition, 130*, 503-506.

- **Van Staveren, W.A., West, C.E., Hoffmans, M.D.A.F., Bos, P., Kardinaal, A.F.M., Van Poppel, G.A.F.C., et al.** (1986). Comparison of contemporaneous and retrospective estimates of food consumption made by a dietary history method. *American Journal of Epidemiology, 123,* 884-893.

- **VandenLangenberg, G.M., Mares-Perlman, J.A., Klein, R., Klein, B.E.K., Brady, W.E., & Palta, M**. (1998). Associations between antioxidant and zinc intake and the 5 year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *American Journal of Epidemiology, 148*, 204–214.
- **Villard, L., & Bates, C.J**. (1987). Dietary intake of vitamin A precursors by rural Gambian pregnant and lactating women. *Human Nutrition, 41*, 135-145.

- **Vingerling, J.R., Dielemans, I., Bots, M.L., Hofman, A., Grobbee, D.E., & de Jong, P.T.** (1995a). Age-related macular degeneration is

associated with atherosclerosis. The Rotterdam Study. *American Journal of Epidemiology, 142,* 404-409.

- **Vingerling, J.R., Dielemans, I., Hofman, A., Grobbee, D.E., Hijmering, M., Kramer, C.F., et al.** (1995b). The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology, 102,* 205-210.

- **Visioli, F., & Galli, C**. (2001). The role of antioxidants in the Mediterranean diet. *Lipids, 36*, S49-S52.

- **Vitale, S., West, S., Hallfrisch, J., Alston, C., Wang, F., Moorman, C., et al.** (1993). Plasma antioxidants and risk of cortical and nuclear cataract. *Epidemiology, 4,* 195-203.

- **Vu, H.T.V., Robman, L., Hodge, A., McCarty, C.A., & Taylor, H.R.** (2006). Lutein and zeaxanthin and the risk of cataract: the Melbourne visual impairment project. *Investigative Ophthalmology and Visual Science, 47,* 3783-3786.

- **Weintraub, J.M., Willett, W.C., Rosner, B., Colditz, G.A., Seddon, J.M., & Hankinson, S.E.** (2002). A prospective study of the relationship between body mass index and cataract extraction among US women and men. *International Journal of Obesity, 26,* 1588-1595.
- **West, S., Vitale, S., Hallfrisch, J., Muńoz, B., Muller, D., Bressler, S., et al.** (1994). Are antioxidants or supplements protective for age-related macular degeneration? *Archives of Ophthalmology, 112,* 222-227.

- **Weststrate, J. A., & Meijer, G. W.** (1998). Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly

hypercholesterolaemic subjects. *European Journal of Clinical Nutrition, 52*, 334–343.

- **Whiting, S.J., & MacKenzie, M**. (1998). Assessing the changing diet of Indigenous Peoples. *Nutrition Reviews, 56*, 248-250.

- **Wingerath, T., Stahl, W., & Sies, H**. (1995). β-cryptoxanthin selectively increases in human chylomicrons upon ingestion of tangerine concentrate rich in β-cryptoxanthin esters. *Archives of Biochemistry and Biophysics, 324*, 385-390.

- **World Health Organization**. (1998). Fortification of flour with iron in countries of the eastern Mediterranean, Middle East and North Africa. Annex 1.

- **World Health Organization**. (2004). *Magnitude and causes of visual impairment.* Retrieved July 10, 2007, from http://www.who.int/ mediacentre/factsheets/fs282/en/.

- **World Health Organization**. (2005). State of the World's sight. Vision 2020: The right to sight. 1999-2005.

- **Wyszecki, G., & Stiles, W.S**. (1982). *Color Science*. New York: Wiley.

- **Xu, L., Cui, T., Zhang, S., Sun, B., Zheng, Y., Hu, A., et al.** (2006). Prevalence and risk factors of lens opacities in urban and rural Chinese in Beijing. *Ophthalmology, 113,* 747-755.

- **Yemelyanov, A.Y.U., Katz, N.B., & Bernstein, P.S.** (2001). Ligand-binding characterization of xanthophyll carotenoids to solubilized

membrane proteins derived from human retina. *Experimental Eye Research, 72,* 381-392.

- **Yeum, K.J., Shang, F.M., Schalch, W.M., Russell, R.M., & Taylor, A.** (1999). Fat-soluble nutrient concentrations in different layers of human cataractous lens. *Current Eye Research, 19*, 502-505.

- **Yeum, K.J., Taylor, A., Tang, G., & Russell, R.M**. (1995). Measurement of carotenoids, retinoids, and tocopherols in human lenses. *Investigative Ophthalmology & Visual Science, 36*, 2756-2761.

- **Younan, C., Mitchell, P., Cumming, R.G., Panchapakesan, J., Rochtchina, E., & Hales, A.M.** (2002). Hormone replacement therapy, reproductive factors, and the incidence of cataract and cataract surgery: the Blue Mountains Eye Study. *American Journal of Epidemiology, 155,* 997-1006.

- **Young, A.J, & Lowe, G.M**. (2001). Antioxidant and prooxidant properties of carotenoids. *Archives of Biochemistry and Biophysics, 385*, 20-27.

- **Zeitlin, M.F., Megawangi, R., Kramer, E.M., & Armstrong, H.C**. (1992). Mothers' and children's intakes of vitamin A in rural Bangladesh. *American Journal of Clinical Nutrition, 56*, 6136-6147.

8.0 APPENDICES

Appendix 1

Consent Form for Study Participants

Dietary Habits and Age-Related Cataracts in a Rural Lebanese Elderly Population

Informed consent

The American University of Beirut, in collaboration with McGill University, Canada, is conducting a study on the relationship between the consumption of leafy greens and age-related cataracts in a rural elderly Lebanese population. The study will be carried out by Joelle Zeitouny, a graduate student at McGill University, Canada, under the supervision of Dr Timothy Johns of the School of Dietetics and Human Nutrition, in collaboration with Dr Malek Batal from the Department of Nutrition and Food Science at the American University of Beirut.

The study aims at better understanding the relationship between the consumption of leafy greens and age-related cataracts and will be carried out in the northeast of Lebanon during the months of June and July among participants who are 45 years and above, and native and resident of the villages where the study will take place. Cases will be selected through health centers in the Hermel region and will be matched to controls of the same age, gender, gender and region but with no chronic eye disease. Joelle Zeitouny, with the help of three interviewers from the region, will take your body weight and height and your mid-upper arm circumference and will carry out two interviews will you. The first one involves answering a general questionnaire about your socio-economic status, health and diet, as well as a 7-day semi-quantitative food frequency questionnaire. The second interview involves answering a 3-month food frequency questionnaire only. The interviews will be held on a one to one basis. Joelle Zeitouny or/and any of the other interviewers will sit with you for a period of 30-45 minutes to conduct the questionnaires, take the above-cited measurements, and provide any explanation or clarification if necessary.

Participation is voluntary and you are not required to participate. You have the right to refuse to answer any of the questions or to forbid the researcher to take any of the above-cited measurements. You may withdraw from the study at any point and for any reason without any penalty or prejudice.

The information you will provide in the questionnaires will be kept strictly confidential. This information will be used solely by the investigators conducting the study. You will be referred to by a numbered code on the questionnaire. The data will be combined to give general statements about the consumption of leafy greens and the occurrence of age-related cataracts and will not be attributed to you specifically in any publication resulting from this study.

The study does not present any harm or risk to you. In case any adverse event occurs as a result of the study, there will be no compensation to cover the expenses. The study should lead to a better understanding of the role of the consumption of leafy greens in preventing age-related cataracts and should promote the consumption of wild leafy

greens. A copy of the results will be translated and presented to the community of Hermel.

Consent of the investigator:

I have explained to the participant the study in detail including the proceedings and any disadvantages. I have answered all questions clearly to the best of my abilities.

_____	_____	_____
(Investigator's Name)	(Investigator's Signature)	(Date)

Consent of the participant:

I have read this letter of consent and understood its content. All my questions have been answered. Accordingly I hereby agree to participate in this study, and I understand that the investigators will stand ready to answer my questions and I can contact them on 01-350000 ext: 4457. I can also contact the Institutional Review Board for human rights at 01-350 000 ext: 4911 in case the answers needed more clarifications. I also understand fully that I am free to withdraw from this study at any time.

_____	_____	_____
(Participant's Name)	(Participant's Signature)	(Date)

موافقة للإشتراك في بحث علمي

ألعادات الغذائية و الماء الزرقاء عند المتقدمين في السن في الريف اللبناني

إن الجامعة الأميركية في بيروت بالتعاون مع جامعة مكغيل في كندا، تقوم ببحث علمي حول ارتباط ا ستهلاك الأعشاب البرية الصالحة للأكل بالماء الزرقاء عند المتقدمين في السن في القرى اللبنانية. ستقوم بانجاز هذه الدرا سة الانسة جوال زيتوني، طالبة درا سات عليا في جامعة مكغيل في كندا ، تحت ا شرا ف د. تيموثي جونز من كلية علوم الحمية والتغذية البشرية، بالتعاون مع د. مالك بطل من دائرة التغذية و العلوم الغذائية في الجامعة الاميركية في بيروت. يعاونها في هذه المهمة ثلاثة محاورين من المنطقة.

يهدف هذا البحث إلى التحقق من المفعول الوقائي المحتمل لأوراق الخضار الخضراء (خاصة النباتات البرية الصالحة للأكل) على داء الماء الزرقاء عند سكان الريف في منطقة الهرمل في لبنان خلال شهور ايا ر و حزيران و تموز ٢٠٠٦. يجب على المشاركين ان يكونوا في سن ال45 و ما فوق ، من مواليد و سكان القرى التي يجري فيها البحث . هذه الدراسة ستنتقي الحالات من العيادات المتخصصة في المنطقة. ستقوم الانسة جوال زيتوني او اي من المحاورين باخذ وزنكم و طولكم وقياس محيط نصف الذراع و باجراء مقابلتين معكم. تتضمن المقابلة الاولى الاجابة على استما رة تتمحور حول وضعكم الاجتماعي والاقتصا دي ، صحتكم العامة وعا دا تكم الغذائية كما عن وتيرة تكرار الاطعمة لديكم خلال الاسبو ع الماضي . اما المقابلة الثانية فستتضمن الاجابة على استمارة عن وتير ة تكرار الاطعمة لديكم خلال الاشهر الثلاثة الماضية . ستجر ى المقابلات شخصيا" مع الانسة جوا ل زيتوني او اي من المحاورين لمدة تتراوح بين 30 و 45 دقيقة لتعبئة الاستمارة ولاعطاء اي إيضاحات أو معلومات إضافية عن أي موضوع مذكور في الإستمارة أو عن الدراسة ككل.

الاشتراك اختياري ولا يمكن اجباركم على المشاركة . لديكم الحق في رفض الاجابة عن اي من الاسئلة او في منع الباحث من اخذ اي من القياسات المذكو ر ة اعلاه. كما يكمنكم الانسحاب من الدر اسة في اي و قت ولاي سبب من دون اي جزاء او اجحاف.

إن جميع المعلومات التي ستجمع خلال هذه المقابلة سيكون استعمالها بحثي بحت ولن يتم استعمالها لأي غرض ثان. في حال وافقتم على المشاركة في هذه الدراسة، سيبقى إسمكم طي الكتمان . سيتم الاشارة اليكم برقم رمزي موضوع على الاستمارة . لن يتم ذكر اسمكم في أي نشرة ناتجة عن هذه الدراسة.

ليس هناك أي تأثيرات سلبية من جراء هذا البحث. إن هذا البحث سيمكننا من ربط العادات الغذائية التقليدية (تحديداً الأعشاب البرية الصالحة للأكل) بداء الماء الزرقاء الناتجة عن التقدم بالسن ، إذ أننا نأمل اكتشاف فوائد استهلاك الأعشاب البرية على داء الماء الزرقاء الناتجة عن التقدم بالسن. في حال وقوع أي حادث أو مشكلة من جراء الدراسة، لن يكون هناك أي تعويض لتغطية النفقات أياً كان نوعها. في النهاية، سيتم اعطاء نسخة عن خلاصة الدراسة الى سكان منطقة الهرمل.

موافقة الباحث

لقد شرحت بالتفصيل للمشترك في البحث طبيعته ومجرياته وتأثيراته السلبية. ولقد أجبت على كل أسئلته بوضوح على خير ما أستطيع.

_____ _____ _____

إسم الباحث او الشخص المولى توقيع الباحث او الشخص المولى التاريخ

موافقة المشترك

قرأت استمارة القبول هذه وفهمت مضمونها. تمت الأجابة على أسئلتي جميعها. بناء عليه إنني أوافق على الإشتراك في هذه الدراسة، وإني أعلم ان الباحثين سيكونون مستعدين للإجابة على أسئلتي، وأنه باستطاعتي الإتصال بهم على الهاتف 01-350000 المقسم 4457 أو بلجنة الأخلاقيات على الهاتف 01-350000 المقسم 4911 . كما أعرف تمام المعرفة بانني حر في الإنسحاب من هذا البحث متى شئت حتى بعد التوقيع على الموافقة.

_____	_____	_____
إسم المشترك	توقيع المشترك	التاريخ

Appendix 2

Socio-demographic Questionnaire

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

American University of Beirut

Department of Nutrition and Food Science

Dietary habits and Age-Related Cataracts in a Rural Lebanese Elderly Population

1	**Name of the interviewer:**	
2	**Gender of the interviewer:**	□ **Male** □ **Female**
3	**Code of the clinic:**	
4	**Start of the interview:**	**(Please write down the time) A.M. / P.M.**
5	**Date of the interview:**	/ / **Year Month Day**
6	**Number of the questionnaire:**	
7	**Name of the interviewee:**	**First name:** _____ **Family name:** _____ **Father's name:** _____
8	**Was the interviewee diagnosed with age-related cataracts?**	**1. Yes** **2. No**

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

Measurements

(It's preferable to take the measurements after the completion of the questionnaire):

1. Weight (kg):
2. Height (m):
3. Mid-upper arm muscle circumference (cm):

General information

1. Gender: □ Male □ Female

2. Age:

3. Iris color: 1 □ Blue or green 2 □ Hazel, brown or black

4. Place of birth: a. Country: b. Village:
c. Governorate: d. District:

5. Place of residence: a. Village: b: Governorate:
c. District:

6. Since when do you live in or visit the village?
a. Since birth b. Since childhood (less than 12 years old)
c. Since adolescence (12-19 years old) d. Between 20 to 60 years old
e. After 60 years old

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

7. Marital status: 1 □ Never married 2 □ Married
3 □ Separated 4 □ Divorced
5 □ Widowed

8. Education level:

1 □ No schooling (illiterate)
2 □ No schooling (reads and writes)
3 □ Elementary school
4 □ Intermediate school
5 □ High School
6 □ University
7 □ Technical
8 □ Other: _____

9. Present occupation: Specify:_____

1 □ Farmer
2 □ Employee (blue collar)
3 □ Employee (white collar)
4 □ Self-employed (specify): _____
5 □ Unemployed (looking for a job)
6 □ Unemployed (not looking for a job)
7 □ Homemaker
8 □ Retired (specify past occupation): _____
9 □ Other (specify): _____

10. Number of hours spent daily at work:

1 2 3 4 5 6 7 8 9 more NA

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

11. Number of working days per week:

1 2 3 4 5 6 7 NA

12. Number of hours of sun exposure per day:

1 2 3 4 5 6 7 8 9 10 11 12 more

13. Generally speaking, how do you regard your household's economic situation in relation to people who live in Lebanon?

1 □ We are among the well-offs in Lebanon
2 □ We are middle-class, neither rich nor poor
3 □ We are poor

14. If your household had a sudden need for 150,000 LBP would you be able to raise the money in a week?

1 □ Yes, would resort to my savings
2 □ Yes with some help from others
3 □ Yes by taking advance on my salary
4 □ Yes we would sell our belongings
5 □ No impossible
6 □ I don't know
7 □ No answer

Health status

1. Have you ever been diagnosed with age-related cataract?

1 □ Yes 2 □ No

2. If yes, did you have cataract extraction?

1 □ Yes 2 □ No

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

3. Has any member of your family been diagnosed with age-related cataract?
1 □ Yes 2 □ No

4. Have you been diagnosed with another eye disease?
1 □ Yes (Specify:_____) 2 □ No

5. Do you currently smoke?
1 □ Yes 2 □ No
6. If no, were you a previous smoker?
1 □ Yes 2 □ No

7. Do you smoke the narguileh?
1 □ Yes 2 □ No

8. If no, were you a previous narguileh smoker?
1 □ Yes 2 □ No

9. On a scale of 1 to 10 how do you rate your level of stress in everyday life (10 being the most stressful and 1 being least stressful):

1 2 3 4 5 6 7 8 9 10

Diet information

1. Do you usually collect wild edible leafy greens?
1 □ Yes 2 □ No

2. If yes, when do you collect these plants:
1 □ January 2 □ February 3 □ March 4 □ April 5 □ May 6 □ June 7 □ July

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

3. How many times per season do you collect wild leafy greens?

1 2 3 4 5 6 7 8 9 10 more

4. What do you do with them? (percentage added up to 100%)

a. Consume them: b. Give to others (family, friends, others): c. Sell:

5. How did you learn about wild leafy greens (collection, growing and information)?

1 □ From my parents
2 □ From my grandparents
3 □ From my siblings
4 □ From extended family members
5 □ From expert in village
6 □ From school/university
7 □ Other (specify):_____

6. In your opinion, what are the benefits of wild leafy greens?

7. Are you taking any vitamin/mineral supplement?

1 □ Yes 2 □ No

8. If yes, please specify: Kind:_____

# of pills per day:_____

# of pills per week:_____

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

9. Are you taking any medication?
1 □ Yes 2 □ No

10. If yes, please specify: Kind:_____
# of pills per day:_____
# of pills per week:_____

Appendix 3

Semi-quantitative Food Frequency Questionnaires

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee	

Food frequency questionnaire

How frequently do you consume these foods?

Type of food	Amount	Number of times			
		# over the last 7 days	# over the last 3 months		
			# per day	# per week	# per month
1. Breads and cereals					
1.1 Bread (whole wheat)	1 slice (32g)				
1.2 Bread (white)	1 slice (30g)				
1.3 Burghol	1 cup (182g)				
1.4 Corn	½ cup (82g)				
1.5 Rice (white)	1 cup (186g)				
1.6 Pasta	1 cup (140g)				
1.7 Cooked wheat	1 cup (150g)				
2. Fruits					
2.1 Apricots	3 items (105g)				
2.2 Grapes	10 items (24g)				
2.3 Loquats	3 items (48g)				

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

Type of food	Amount	Number of times			
		# over the last 7 days	# over the last 3 months		
			# per day	# per week	# per month
2.4 Mango	1 item (207g)				
2.5 Nectarine	1 item (142g)				
2.6 Peach	1 item (150g)				
2.7 Persimmons	1 item (168g)				
3. Vegetables					
3.1 Carrot	½ cup (64g)				
3.2 Chicory	½ cup (14.5g)				
3.3 Coriander	½ cup (8g)				
3.4 Cucumber	1 item (52g)				
3.5 Eggplant	1 cup (99g)				
3.6 Lettuce	½ cup (23.5g)				
3.7 Mint	½ cup (45.6g)				
3.8 Okra	1 cup (160g)				
3.9 Parsley	½ cup (30g)				

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee	

Type of food	Amount	Number of times			
		# over the last 7 days	# over the last 3 months		
			# per day	# per week	# per month
3.10 Pea (green)	½ cup (85g)				
3.11 Pepper green	1 item (119g)				
3.12 Pepper red	1 item (119g)				
3.13 Potato	1 item (167g)				
3.14 Pumpkin	½ cup(122.5g)				
3.15 Spinach	½ cup (90g)				
3.16 Sweet potato	1 item (151g)				
3.17 Swiss chard	½ cup (87.5g)				
3.18 Tomato	1 item (123g)				
3.19 Zucchini	½ cup (90g)				
3.20 Rocket	½ cup (10g)				
3.21 Reched	½ cup (8g)				
3.22 Tomato paste	1 tbsp (16g)				
3.23 Ketchup	1 tbsp (15g)				

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

Type of food	Amount	Number of times			
		# over the last 7 days	# over the last 3 months		
			# per day	# per week	# per month
4. Beans					
4.1 Chickpeas	1 cup (164g)				
4.2 Faba beans	1 cup (170g)				
4.3 Green beans (loubieh msallet)	½ cup (62.5g)				
4.4 Runner beans (loubieh baidrieh)	½ cup (62.5g)				
4.5 Lima beans	1 cup (170g)				
4.6 Long beans (fasolia baida)	1 cup (179g)				
4.7 Lentils	1 cup (198g)				
5. Milk and dairy products					
5.1 Milk whole	1 cup (244g)				
5.2 Laban whole	1 cup (245g)				
5.3 Labneh whole	2 tbsp (29g)				
5.4 Cheese (all kinds)	1 ounce (28g)				
5.5 Kichek	½ cup (122g)				

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

Type of food	Amount	Number of times			
		# over the last 7 days	# over the last 3 months		
			# per day	# per week	# per month
5.6 Chanklich	½ cup (113g)				
6. Meats and eggs					
6.1 Eggs (yolk)	1 item (17g)				
6.2 Eggs (whole)	1 item (50g)				
6.3 Organ meats	3 ounces (84g)				
6.4 Red meat	3 ounces (84g)				
6.5 Chicken	3 ounces (84g)				
6.6 Fish	3 ounces (84g)				
6.7 Birds and other wild animals	3 ounces (84g)				
7. Fats and oils					
7.1 Olives	6 items(19.2g)				
7.2 Olive oil	1 tbsp (13.5g)				
7.3 Butter	1 tsp (5g)				
7.4 Ghee	1 tsp (5g)				

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

Type of food	Amount	Number of times			
		# over the last 7 days	# over the last 3 months		
			# per day	# per week	# per month
8. Drinks					
8.1 Orange juice (100% natural)	½ cup (124g)				
8.2 Other kinds of natural juices					
	½ cup				
	½ cup				
9. Wild edible leafy greens					
9.1 Bakleh barrieh (Purslane)	½ cup (21.5g)				
9.2 Choumar (Fennel)	½ cup (4.5g)				
9.3 Hindbeh barrieh (Wild Chicory)	½ cup (52.5g)				
9.4 Hommayda (Sorrel)	½ cup (52.5g)				
9.5 Dardarieh (Fraxinella)	½ cup (52.5g)				
9.6 Jarjeer (Watercress)	½ cup (17g)				
9.7 Khebbayzeh (Mallow)	½ cup (52.5g)				
9.8 Korsanneh (Eryngo)	½ cup (52.5g)				

Code of the interviewer	Code of the clinic	Gender of interviewee	Code of interviewee

Type of food	Amount	Number of times			
		# over the last 7 days	# over the last 3 months		
			# per day	# per week	# per month
9.9 Mokho bi ebbo ( )	½ cup (52.5g)				
9.10 Thyme	½ cup (19.2g)				
9.11 Asparagus	½ cup (90g)				
9.12 Kotayfeh	½ cup (66g)				
9.13 Other wild leafy greens					
	½ cup				
	½ cup				

Comments:

End of the interview: _____ Time the interview took: _____

Appendix 4

McGill University's Certificate of Ethical Approval

MCGILL UNIVERSITY
FACULTY OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES

CERTIFICATE OF ETHICAL ACCEPTABILITY FOR RESEARCH INVOLVING HUMANS

The Faculty of Agricultural and Environmental Sciences Research Ethics Board consists of 4 members nominated by the Faculty of Agricultural and Environmental Sciences Nominating Committee and elected by Faculty, an appointed member from the community and an individual versed in ethical issues.

The undersigned considered the application for certification of the ethical acceptability of the project entitled:

Effect of non-nutrient constituents on the incidence of age-related cataracts in poor rural Lebanon

as proposed by:

Applicant's Name Joelle Zeitouny Faculty Supervisor's Name Dr. Timothy [illegible]

Applicant's Signature [signature] Faculty Supervisor's Signature [signature]

Degree / Program / Course MSc Human Nutrition (Thesis) Granting Agency

Grant Title:
(If different from project title)

For administrative use REB # 867-0406

Approval Period: June 14, 2006 to June 13, 2007

The application is considered to be:

A Full Review ____ An Expedited Review X

Approval:

STAN KUBOW
Chair, Research Ethics Board, FAES

[signature] June 14, 2006
Signature Date

To be submitted to:
Chair, Research Ethics Board, Faculty of Agricultural and Environmental Sciences
c/o Lynn Murphy
Macdonald Campus Research Office
Raymond Building, Room R3-032
Tel: (514) 398-8718

September 2005

Appendix 5

American University of Beirut's Certificate of Ethical Approval

لجنة الأخلاقيات
كلية الطب
INSTITUTIONAL REVIEW BOARD
FACULTY OF MEDICINE

AUB American University of Beirut
الجامعة الأميركية في بيروت

To: Dr. Malek Batal
Date: April 05, 2006

Principal Investigator: Dr. Malek Batal
American University Hospital
Protocol Number: NUT.MB.04
Protocol Name: Wild Edible Plant consumption and degenerative Eye disease in a rural Lebanese Elderly Population: A Case-Control Study

Thank you for submitting to the IRB the above named study for review.
The IRB reviewed the study, the English and Arabic informed consent forms (version date March 17, 2006), the English and Arabic Questionnaires in an expedited manner.

This is to grant you approval to the study, the English and Arabic informed consent forms (version date March 17, 2006) and questionnaires for a period of one year from the above date, at which time a progress report is kindly requested from you.

The membership of this Institutional Review Board complies with the membership requirements defined in the US Code of Federal Regulation (21CFR56 and 45CFR46) of the Food and Drug Administration. In addition, the IRB operates in a manner consistent with Good Clinical Practices under the ICH guidelines, with FDA and applicable national/local regulations.

Sincerely,

Ibrahim Salti, MD, PhD
Chairperson of the IRB

cc. Dr. Ali Bazarbachi, Assistant Dean for Research, Faculty of Medicine

Appendix 6

Lutein and Zeaxanthin's Composition of Food Items

Nutritive value of foods. Values of lutein and zeaxanthin, β-carotene, RAE, α-tocopherol and vitamin C as present or entered on CANDAT.

FOOD ITEMS	Lutein and Zeaxanthin (µg/100g)	RAE values (µg/100g)	ß-carotene (µg/100g)	α-tocopherol (mg/100g)	Vitamin C (mg/100g)
1. Breads and cereals					
1.1 Bread (whole wheat)	53	0	0	0.61	0
1.2 Bread (white)	53	0	0	0.3	0
1.3 Burghol (bulgur)	54	0	1	0.01	0
1.4 Corn	1029	4	32	0.04	8.5
1.5 Rice (white)	**	0	0	**	0
1.6 Pasta	31.25 [a]	0	0	0.06	0
1.7 Cooked wheat	2750 [b]	0	0	1.22 [c]	0
2. Fruits					
2.1 Apricots	89	96	1094	0.89	10
2.2 Grapes	72	3	39	0.19	10.8
2.3 Loquats	126 [d]	76	916.2	**	1
2.4 Mango	10 [b]	38	445	1.12	27.7

2.5 Nectarine	130	13	150	0.77	5.4
2.6 Peach	91	16	162	0.73	6.6
2.7 Persimmons	834	21	253	0.73	7.5
3. Vegetables					
3.1 Carrot	687	860	8332	1.03	3.6
3.2 Chicory	10300	286	3430	2.26	24
3.3 Coriander	865	337	3930	2.5	27
3.4 Cucumber	23	5	45	0.03	2.8
3.5 Eggplant	930 [b]	2	22	0.41	1.3
3.6 Lettuce	2312	290	3484	0.13	24
3.7 Mint	1700 [b]	204	2436	0.34 [e]	13.3
3.8 Okra	390	14	170	0.27	16.3
3.9 Parsley	5561	421	5054	0.75	133
3.10 Pea (green)	1350	27	320	0.03	9.6
3.11 Pepper green	341	18	208	0.37	80.4
3.12 Pepper red	51	157	1624	1.58	190
3.13 Potato	9	0	2	0.01	13
3.14 Pumpkin	1014	250	2096	0.8	4.7

3.15 Spinach	11308	524	6288	2.08	9.8
3.16 Sweet potato	0	787	9444	0.94	12.8
3.17 Swiss chard	11015	306	3652	1.89	18
3.18 Tomato	123	42	449	0.54	12.7
3.19 Zucchini	1150	56	670	0.12	4.6
3.20 Rocket	3555	119	1424	0.43	15
3.21 Reched ( )	5101.15 [f]	306 [g]	4207.46 [f]	2.09 [f]	50 [g]
3.22 Tomato paste	0	76	901	4.3	21.9
3.23 Ketchup	210 [h]	47	560	1.46	15.1
4. Beans					
4.1 Chickpeas	440 [b]	1	16	0.35	1.3
4.2 Faba beans	654 [b]	1	9	0.02	0.3
4.3 Green beans	555 [b]	0	0	**	1.2
4.4 Runner beans	709	35	420	0.45	9.7
4.5 Lima beans	360 [b]	0	0	0.18	0
4.6 Long beans	410 [b]	0	0	0.97	0
4.7 Lentils	0	0	5	0.11	1.5

5. Milk and dairy products					
5.1 Milk whole	**	33	24	**	1.5
5.2 Yoghurt whole	**	43	31.46	**	0.53
5.3 Labneh whole	**	162 [g]	**	**	0 [g]
5.3 Cheese (all kinds)	0	198	63	0.34	0
5.4 Kichek	**	26 [g]	**	**	0 [g]
5.5 Chanklich	**	360 [g]	**	**	0 [g]
6. Meats and eggs					
6.1 Eggs (yolk)	1576 [b]	381	0	6.03	0
6.2 Eggs (whole)	338 [b]	140	0	1.88	0
6.3 Organ meats	0	4968	232	0.38	1.3
6.4 Red meat	**	0	0	**	0
6.5 Chicken	**	28	0	0.3	2.7
6.6 Fish	0	17	0	0.2	0.5
6.7 Birds and other wild animals	**	73	0	**	6.1
7. Fats and oils					

7.1 Olives	510	20	237	1.65	0.9
7.2 Olive oil	350 [i]	0	0	14.35	0
7.3 Butter	0	684	158	2.32	0
7.4 Ghee	**	0	0	**	0
8. Drinks (Natural)					
8.1 Orange juice	115	10	33	0.04	50
8.2 Carrot juice	333	775	9303	1.16	8.5
8.3 Apple juice	16	0	0	0.01	0.9
8.4 Lemon juice	9	1	3	0.15	46
9. Wild edible leafy greens					
9.1 Purslane	7000 [i]	66	792	12.2 [j]	21
9.2 Fennel	3669 [k]	353.62 [f]	1196 [k]	0.63 [k]	101 [k]
9.3 Wild Chicory	10300	286	3430	2.26	24
9.4 Sorrel	3438 [k]	174	2082	0.85 [k]	26.3
9.5 Dardarieh (*Centaurea pallescens*)	5101.15 [f]	353.62 [f]	4207.46 [f]	2.09 [f]	30.65 [f]
9.6 Watercress	5767	235	2820	1	43

9.7 Mallow	5101.15 [f]	353.62 [f]	4207.46 [f]	2.09 [f]	165 [l]
9.8 Eryngo	5101.15 [f]	353.62 [f]	4207.46 [f]	2.09 [f]	30.65 [f]
9.9 Mokho bi ebbo ( )	5101.15 [f]	353.62 [f]	4207.46 [f]	2.09 [f]	30.65 [f]
9.10 Thyme	5101.15 [f]	238	2851	2.5 [m]	160.1
9.11 Asparagus	771	50	604	1.5	7.7
9.12 Amaranth	13000 [i]	139	1662	2.09 [f]	41.1
9.13 Akkoub (*Gundelia tournefortii*)	5101.15 [f]	353.62 [f]	4207.46 [f]	2.09 [f]	30.65 [f]
9.14 Grape leaves	1747	1376	16194	2	11.1
9.15 Jew's mallow	1747	259	3111	0.7	33
9.16 Corn poppy	1154 [k]	353.62 [f]	750 [k]	1.37 [k]	17 [k]
9.17 Msabb el zeit ( )	5101.15 [f]	353.62 [f]	4207.46 [f]	2.09 [f]	30.65 [f]

** Data not available.

[a] Humphries, J.M., & Khachik, F. (2003). Distribution of lutein, zeaxanthin, and related geometrical isomers in fruit, vegetables, wheat, and pasta products. *Journal of Agricultural and Food Chemistry, 51*, 1322-1327.

[b] Calvo, M.M. (2005). Lutein: a valuable ingredient of fruit and vegetables. *Critical Reviews in Food Science and Nutrition, 45*, 671-96.

[c] Hidalgo, A., Brandolini, A., Pompei, C., & Piscozzi, R. (2006). Carotenoids and tocols of einkorn wheat (*Triticum monococcum* ssp. *monococcum* L.). *Journal of Cereal Science, 44*, 182-193.

[d] Gross, J., Gabai, M., & Lifshitz, A. (1973). Carotenoids of *Eriobotrya japonica*. *Phytochemistry, 12*, 1775-1782.

[e] Gómez-Coronado, D.J.M., Ibañez, E., Rupérez, F.J., & Barbas, C. (2004). Tocopherol measurement in edible products of vegetable origin. *Journal of Chromatography A, 1054*, 227-233.

[f] Average nutritive value of leafy greens (please see table below).

[g] Pellett, P.L., & Shadarevian, S. (1970). *Food composition tables for use in the Middle East*. Beirut: American University of Beirut.

[h] Heinonen, M.I., Ollilainen, V., Linkola, E.K., Varo, P.T., & Koivistoinen, P.E. (1989). Carotenoids in Finnish foods: Vegetables, fruits, and berries. *Journal of Agricultural and Food Chemistry, 37*, 655-659.

[i] Su, Q., Rowley, K.G., Itsiopoulos, C., & O'Dea, K. (2002). Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: green leafy vegetables, figs and olive oil. *European Journal of Clinical Nutrition, 56,* 1149-1154.

[j] Simopoulos, A.P., Norman, H.A., Gillaspy, J.E., & Duke, J.A. (1992). Common purslane: a source of omega-3 fatty acids and antioxidants. *Journal of the American College of Nutrition, 11*, 374-382.

[k] Vardavas, C.I., Majchrzak, D., Wagner, K.-H., Elmadfa, I., & Kafatos, A. (2006). The antioxidant and phylloquinone content of wildly grown greens in Crete. *Food Chemistry, 99*, 813-821.

[l] Habegger, R., & Puschmann, G. (1992). *Malva neglecta* (Wallr.). *Acta Horticulturae, 318*, 135-141.

[m] Lagouri, V., & Boskou, D. (1996). Nutrient antioxidants in oregano. *International Journal of Food Science and Nutrition, 47*, 493-497.

Average nutritive value of leafy greens.

FOOD ITEMS	**Energy** (Kcal/100g)	**Lutein and Zeaxanthin** (µg/100g)	**RAE values** (µg/100g)	**ß-carotene** (µg/100g)	**α-tocopherol** (mg/100g)	**Vitamin C** (mg/100g)
1. Chicory	23	10300	286	3430	2.26	24
2.Coriander	23	865	337	3930	2.5	27
3. Lettuce	17	2312	290	3484	0.13	24
4. Mint	44	1700 [a]	204	2436	0.34 [b]	13.3
5. Parsley	36	5561	421	5054	0.75	133
6. Spinach	23	11308	524	6288	2.08	9.8
7. Swiss chard	20	11015	306	3652	1.89	18
8. Rocket	25	3555	119	1424	0.43	15
9. Purslane	16	7000 [c]	66	792	12.2 [d]	21
10. Sorrel	20	3438 [e]	174	2082	0.85 [e]	26.3
11. Watercress	11	5767	235	2820	1	43
12. Grape leaves	93	1747	1376	16194	2	11.1
13. Jew's mallow	37	1747	259	3111	0.7	33
AVERAGE (± SD)	**29.85** (± 21.1)	**5101.15** (± 3760.9)	**353.62** (± 329.8)	**4207.46** (± 3877.6)	**2.09** (± 3.1)	**30.65** (± 32.1)

[a] Calvo, M.M. (2005). Lutein: a valuable ingredient of fruit and vegetables. *Critical Reviews in Food Science and Nutrition, 45*, 671-96.

[b] Gómez-Coronado, D.J.M., Ibañez, E., Rupérez, F.J., & Barbas, C. (2004). Tocopherol measurement in edible products of vegetable origin. *Journal of Chromatography A, 1054*, 227-233.

[c] Su, Q., Rowley, K.G., Itsiopoulos, C., & O'Dea, K. (2002). Identification and quantitation of major carotenoids in selected components of the Mediterranean diet: green leafy vegetables, figs and olive oil. *European Journal of Clinical Nutrition, 56,* 1149-1154.

[d] Simopoulos, A.P., Norman, H.A., Gillaspy, J.E., & Duke, J.A. (1992). Common purslane: a source of omega-3 fatty acids and antioxidants. *Journal of the American College of Nutrition, 11*, 374-382.

[e] Vardavas, C.I., Majchrzak, D., Wagner, K.-H., Elmadfa, I., & Kafatos, A. (2006). The antioxidant and phylloquinone content of wildly grown greens in Crete. *Food Chemistry, 99*, 813-821.