OPTIMISING DIETARY MODIFICATION FOR AGE RELATED MACULAR DEGENERATION

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An ageing population has raised the priority of reducing the risk for age-related eye diseases that impair sight and quality of life. Chief among these diseases is age-related macular degeneration (AMD), the leading cause of visual impairment among older adults in the developed world. The dietary xanthophylls lutein and zeaxanthin may be effective at attenuating the risk and/or progression of AMD due to their antioxidant and photo-protective properties in the macula, where they are known as the macular pigment. The macular pigment is entirely of dietary origin therefore it is important that AMD patients adopt appropriate dietary modification.

Currently, there is a lack of information regarding the lutein values of specific xanthophyll containing food sources. A lab based investigation was undertaken to determine the lutein concentrations in kale by high-performance liquid chromatography (HPLC); information was established that may improve knowledge on the climate and post-harvest handling, processing and storage effects. Lutein concentrations in minimally processed kale were significantly lower ($p<0.001$) than that of kale freshly harvested. Domestic cooking and storage also had substantial negative effects on kale lutein levels.

A dietary analysis study and a qualitative based study were conducted to determine dietary habits in AMD patients. In align with previous work, AMD patients were found to be under consuming nutrients regarded as important for their condition. Subjects consumed an average of 1.7 mg of L and Z per day, and calorie intakes were significantly below government DRVs ($p<0.05$). Further investigations suggested that this may be attributed to certain physical and psychosocial barriers. Using the results of the laboratory based study, ready meals were created as a novel intervention to improve diet in this population.

This body of research adds insights into dietary interventions within visually impaired groups, studies embedded may enrich dietary advice in the context of AMD.

Key words: AMD, lutein, zeaxanthin, diet, kale

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CONTENTS

LIST OF ABBREVIATIONS

AMD	Age-related macular degeneration
ARMD	Age-related macular disease
ANOVA	Analysis of variance
AREDS	Age-related eye disease study
ARM	Age-related maculopathy
BMI	Body mass index
DRV	Daily recommended value
g	Gram
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
GA	Geographic atrophy
HPLC	High-performance liquid chromatography
L	Lutein
MP	Macular pigment
MPOD	Macular pigment optical density
Mg	Milligram
NHANES	National Health and Nutrition Examination Survey
MZ	Meso-zeaxanthin
RPE	Retinal pigment epithelium
ROS	Reactive oxygen species
RNI	Reference nutrient intake
Z	Zeaxanthin

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Chapter 1: Introduction

Age-related macular degeneration (AMD) is a multifactorial progressive condition affecting the macula, the central area of the retina in the posterior eye. It is the leading cause of visual impairment and blindness registration in the UK [1] [2]. In 2008 the direct and indirect healthcare costs associated with sight loss were around £6.5 billion in the UK which is likely to increase as the number of people with sight loss increases [3]. Along with the burgeoning population of elderly, the number of people suffering with AMD in the UK is predicted to rise, potentially totalling nearly 700,000 cases by 2020 [2]. In the last decade, tremendous research progress has helped with the understanding of the pathogenesis of the disease and in the development of treatment and preventive strategies. While treatments for neovascular (wet) AMD have been used to limit the progression of the condition and reduce the risk of vision loss, proven treatments for the atrophic (dry) form of AMD are yet to be established. Preventive interventions through dietary modification and supplementation have been the focus of a number of observational and interventional trials, [4-10]. This is in part due to the fact they are more affordable than clinical therapies, do not require specialists for administration and many studies suggest that specific nutritional compounds, such as antioxidants and lipids, may influence the development/progression of AMD with few, if any, adverse effects.

1.1 Pathogenesis of AMD

AMD is the degradation of the macula, a pigmented area at the central part of the retina. Near the centre of the macula is the fovea, a small pit that contains a high density of photoreceptors that are responsible for sharp, high-resolution central vision. This part of the retina is able to receive light signals and rapidly transform such signals into chemical and then electrical signals that are sent to the brain via the optic nerve. The brain then converts these electrical signals into visual images. The characteristics of AMD include the loss of central visual function occurring within days or over many years, depending on the type and severity of AMD.

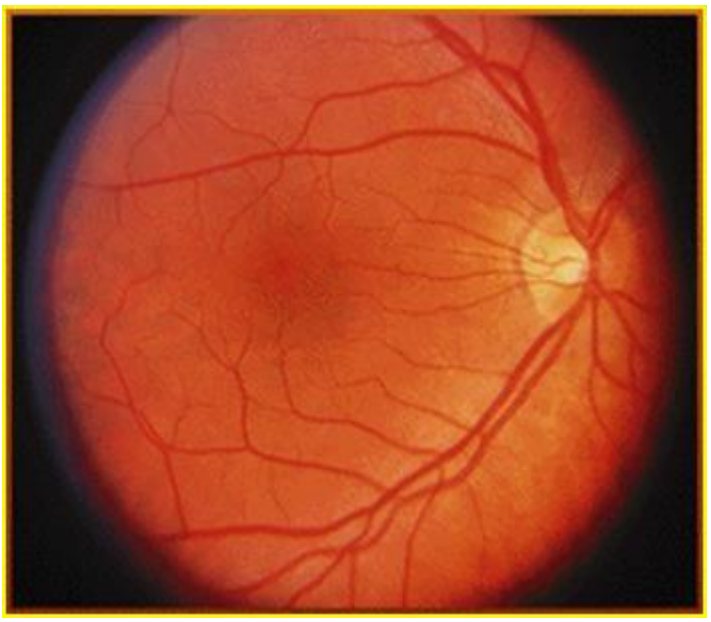

Figure 1.1: Fundus photograph of the back of the eye (source: Aston University staff member, consent obtained).

The following is a fundus photograph of the retina on the interior surface of a healthy eye, showing the macula region and fovea within the shaded area. The retina is the light-sensitive layer lining the inner surface of the eye that contains the photoreceptor rod and cone cells. The fovea is the central area of the macula that contains the highest density of these cells.

If the photoreceptors in the macular region are damaged, as in AMD, the central field of vision is distorted or lost [5]. These photoreceptors are damaged by exposure to extensive oxidative stress in the form of light and oxygen [6] and as a result are persistently shed and turned over. The retinal pigment epithelium (RPE) is responsible for the degradation of the photoreceptor debris and for maintaining the nourishment of the photoreceptors by adequate nutritional support. In the early stages of AMD the combination of inadequate nutritional support and the inability to properly degrade and dispose of cellular debris by the RPE may contribute to the deposition of metabolic debris between the RPE and Bruch's membrane [11]. These lipid deposits are clinically visual as pale yellow spots known as 'drusen' and are the first indicators of early AMD [12], as shown in figure 2.1. In the later stages of the disease, the RPE may atrophy completely. Atrophy can occur in small areas or can be widespread. This form of the disease is known as 'dry AMD' or 'geographic atrophy'. In some cases, new blood vessels (neovascular capillaries) grow under the RPE and occasionally into the sub retinal space. This is known as 'wet' or 'neovascular' AMD

[13]. Haemorrhage can also occur which often results in increased scarring of the retina. The early stages of the disease are in general asymptomatic. In the later stages, there may be considerable distortion within the central visual field leading to a complete loss of central visual function.

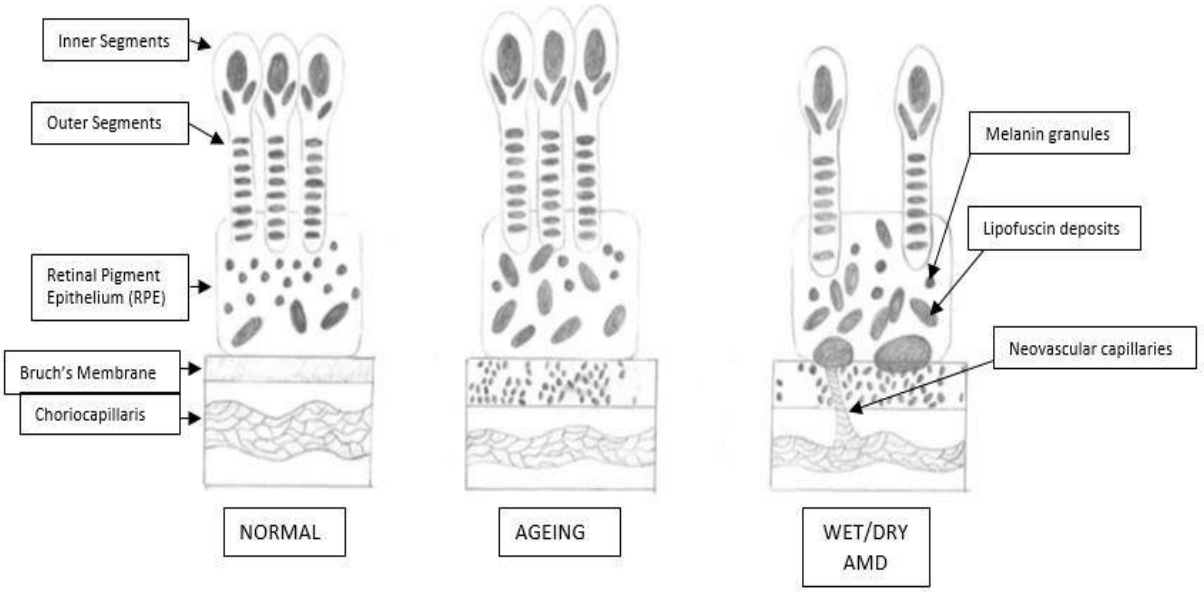

Figure 2.1: Retinal changes of AMD with age.

The figure displays the graphical breakdown of the photoreceptor cells in the macula responsible for the production of high resolution central vision.

Geographic atrophy or dry AMD is the most common form, and is estimated to be present in 15% of eyes by 80 years of age [14], [15], [16]. Progression is slow and legal blindness has been estimated to occur between 5 and 10 years [17]. Neovascular AMD is less common, occurring in 5.2% of the population over 75 years [18], but accounts for 90% of blind registrations [19]. In terms of the demographics of AMD, women have a slightly higher risk for AMD than men. Non-Hispanic blacks have less risk for non-exudative AMD at age 80 than Caucasians, and Asians have a higher rate of non-exudative AMD at age 60 than Caucasians [20, 21]. The three main risk factors for AMD are increasing age [22], [23], [24], [25], smoking [26], [27], and genetic predisposition [28], [29], [30] although other proposed factors include gender [18], race [31], [20], socioeconomic factors [23], cardiovascular disease [23], [28], [32], [33], and as mentioned an area of increasing significance, poor nutrition [34], [35]. These risk factors for AMD have been comprehensively studied in the following reviews [36], [37]. Currently, there are no therapies to treat dry AMD [38] [24]. There are available treatments for some neovascular AMD patients in the form of

photocoagulation, photodynamic therapies or pharmacological approaches by inhibition of the angiogenesis [39]. However, the difficulty in the active management of these therapies has instigated a critical need for the prevention of the onset or progression of AMD through use of dietary antioxidants.

It is thought that people with low systemic antioxidant levels may be more prone to oxidative damage of the retina and therefore, AMD [46]. Oxidation is a natural process of the body and may be defined as the loss of at least one electron when two or more substances interact and produce Reactive Oxygen Species (ROS). Oxidative stress may refer to cellular damage by ROS, which include free radicals, hydrogen peroxide, and singlet oxygen [47]. In a biological context, most ROS are generated as natural by-products of normal cellular metabolism and play vital roles in stimulation of signalling pathways in plant and animal cells in response to changes of intra- and extracellular environmental conditions [40]. ROS are also generated by exogenous sources such as pollutants, tobacco, smoke and radiation. When ROS overwhelm the cellular antioxidant defence system, whether through an increase in ROS levels or a decrease in the cellular antioxidant capacity, oxidative stress occurs [41]. This may result in significant damage to cell structures as these molecules contain one or more unpaired electrons and are rendered unstable and a cytotoxic chain reaction results. Proteins and lipids are significant targets for oxidative attack, and modification of these molecules can increase the risk of mutagenesis [42]. Lipid peroxidation is defined as the process where reactive oxygen species readily attack the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of lipid peroxidation reactions are especially dangerous for the viability of cells and tissues [43]. The retina is particularly susceptible to oxidative stress because of its high consumption of oxygen, high tissue content of polyunsaturated fatty acids and its frequent exposure to visible light [44].

Ocular tissues encompass both low molecular weight antioxidants (ascorbic acid, glutathione and alpha-tocopherol) and high molecular weight antioxidants (catalase, superoxide dismutase, glutathione peroxidase and reductase) which are essential components in the protection against oxidative damage [45]. The eye has a particularly high metabolic rate, and thus an added need for antioxidant protection. Moreover, the natural ageing process decreases the normal production of antioxidants in the ocular media [46], [47, 48]. The macular in particular has a high density of photoreceptor cells which are susceptible to light induced damage. A

photosensitizer is a light-absorbing substance that initiates a photochemical or photophysical reaction. The retina contains a large number of chromophores many of which become photosensitizers when excited by the appropriate wavelength of light [49]. After initial photon absorption, a change in distribution of electrons in the chromophores/photosensitizer molecule occurs and generates an excited singlet state. In this long-lived state, interactions with other intracellular molecules occur generating ROS either via electron transfer (type I photosensitization), or singlet oxygen (type II photosensitization) [50]. These factors have led to an increased interest in the use of dietary antioxidants as a form of added protection against oxidative stress in the retina and subsequent ocular disease [51], [52]. Free radical scavengers such α-tocopherol (vitamin E), ascorbic acid (vitamin C) and lutein (L) and zeaxanthin (Z) are thought to serve as exogenous components of an endogenous defence system that may help to limit light-induced retinal damage [53], [54].

1.2 The role of nutrition in AMD

Evidence supporting an oxidative pathogenesis of AMD and the lack of effective treatment options has increased interest in the potential preventative role of nutritional supplementation or dietary modification. There have been many large and small scale trials which have investigated the link between nutrition and AMD which have yielded positive results. The nutrients associated with the prevention of onset of progression of AMD include the antioxidant vitamins C, E and the vitamin A precursor beta-carotene, the xanthophylls L and Z, omega-3 fatty acids DHA and EPA, zinc, and some B vitamins (folic acid, B2, B3, B6, and B12) [55], [56].

Antioxidant vitamins

With the possibility that long-term exposure to free radicals damages the internal ocular media, diets rich in antioxidants have become of interest with regard to AMD. The antioxidant vitamins which are of particular interest include vitamins A (in the form of beta-carotene), E (in the form of alpha-tocopherol), and C. Beta carotene is a pro-vitamin A carotenoid and the major precursor of vitamin A. Beta-carotene is predominantly found in fruits and vegetables. It is regarded as a powerful antioxidant and has a well-documented role as a quencher of singlet oxygen radicals [57]. Vitamin E, in the form of alpha-tocopherol [58], [59] is predominant in the retina where it is the major lipid-soluble antioxidant of all cellular membranes and lipoproteins [57] [60]. It is highly important in the protection against lipid peroxidation which the eye is particularly susceptible to due to the high concentration of fatty acids [61]. Other roles

of α-tocopherol that are of benefit to ocular health include the maintenance of membrane integrity, lipid metabolism and effective scavenging of ROS [62], [63]. Vitamin C is water-soluble and is involved with several biological processes. It has been shown to have antioxidant properties which enable it to react directly with hydroxyl radicals [64], superoxide [65] and singlet oxygen molecules [66].

The largest trials to investigate the impact of these vitamins combined with other antioxidants, on AMD were the Age-Related Eye Disease Studies (AREDS 1 and 2). This first AREDS trial in 1992 was a long-term multi-centre, prospective study of the clinical course of AMD and age-related cataract. The AREDS trial included a clinical, randomised placebo controlled study to evaluate the effects of high-doses of antioxidant vitamins and zinc on the progression of AMD and vision loss. AREDS participants were 55 to 80 years of age at enrolment and had to be free of any illness or condition that would make long-term follow-up or compliance with study medications unlikely or difficult. On the basis of fundus photographs graded by a central reading centre, best-corrected visual acuity and ophthalmologic evaluations, 4,757 participants were enrolled in one of several AMD categories (table 1.1). The participants' stages of disease ranged from early signs of AMD in one or both eyes such as the presence of small of medium size drusen, to advanced AMD with vision loss in one eye but good vision (at least 20/30) in the other eye.

Category 1	**Category 2**	**Category 3**	**Category 4**
No AMD	*Early AMD*	*Intermediate AMD*	*Advanced AMD*
A few small or no drusen in either eye	Several small drusen or a few medium sized drusen in both eyes	Many medium-sized drusen or one or more large drusen in one or both eyes	In one eye only, either a break-down of photoreceptor cells and supporting tissue in the central retinal area (advanced dry form), or abnormal and fragile blood vessels under the retina (wet form)

Table 1.1 AREDS 1 study categories

This first AREDS trial found that people with the intermediate stage of dry AMD or advanced AMD in one eye had a 25% relative risk reduction of developing advanced AMD over 5 years after taking a supplement which contained beta-carotene (15mg), vitamin C (500mg), vitamin E (400IU), zinc (80mg) and copper (2mg). However, the absolute risk reduction in progression to advanced AMD was 8%, from 28% in the placebo group compared to 20% in the group taking the AREDS formula. The small amount of copper was added to the supplement as high doses of zinc can reduce the levels of copper in the body [8]. The relative risk of losing vision of three or more lines

was reduced by 19% with this combination treatment and the absolute risk reduction for visual acuity loss was 6%. For those study participants who either had no AMD or early AMD, the nutritional combination did not provide an apparent benefit. It was concluded from the study that those with extensive intermediate drusen, large drusen, or non-central geographic atrophy (GA) in one or both eyes, or visual acuity <20/32 attributable to AMD in one eye and without contraindications such as smoking, should consider taking a supplement of antioxidants plus zinc at the dosages used in the trial [8]. Although the AREDS 1 trial gave some promise to nutritional therapy combatting the progression of AMD, the safety of the formula was questioned due to the high doses of nutrients. The UK government recommends that the daily values of these nutrients for those over the age of 50 years should be 40 mg per day for vitamin C, 10 mg per day for vitamin E, 15 mg per day for beta-carotene and 9.5 mg per day for zinc. Table 1.2 displays the combination amounts of nutrients in the AREDS formula in comparison with the UK Reference Nutrient Intakes (RNI's). The RNI values for protein, vitamins and minerals are set for each age/sex group at a level of intake considered likely to be sufficient to meet the requirements of 97.5% of the population.

These UK dietary guidelines are based on the 1991 Report on Dietary Reference Values (DRVs) and set up by the Committee on Medical Aspects of Food Policy (COMA). They include DRVs for energy, protein, fats, sugars, starches, non-polysaccharides (NPS) and 13 vitamins and 18 minerals [67]. The panel found no single criterion to define requirements for all nutrients, so the recommendations are based upon reliable experimental, associations and epidemiological data. For most nutrients, the panel found insufficient data to establish any of these DRVs with great confidence. Thus hypothetical judgements had to be made due to the uncertainties relating to the appropriate parameter by which to assess the requirement and the questionable accuracy of dietary intake data. These references are the current measure until superseded by more rigorous validity studies. The Scientific Advisory Committee on Nutrition (SACN) is currently reviewing some of these nutrient recommendations. The DRV's are deployed in a variety of ways in practice, such as indexes for surveys (EAR), guidance of dietary composition (RNI), for food labels (EAR) and for provision of a general guide in assessing the adequacy of an individual's diet (LRNI/RNI).

AREDS 1		Reference Nutrient Intake (RNI)	
Nutrient	**Amount (mg)**	**Men**	**Women**
Vitamin C	500	40	40
Vitamin E	273	10	10
Beta-carotene	15	15	15
Zinc	80	9.5	7

Table 2.1: AREDS 1 formulation and the corresponding reference nutrient intakes (RNIs) for men and women.

The investigators of the AREDS 1 trial found marginal evidence to suggest there was an increased risk of genitourinary complications in subjects due to the high doses of zinc, (7.5% of subjects) [8]. Similarly, the safety of beta-carotene has been questioned in other studies. Two large randomised controlled trials indicated that smokers who take beta-carotene may be at an increased risk of developing lung cancer [68], [69]. In people with heart disease or diabetes taking high dose vitamin E supplements may also increase their risk of heart failure [70]. The follow up AREDS 2 trial published in 2013 was designed to evaluate the effects of lutein, zeaxanthin and omega 3 as additional ingredients to the original AREDS formula. It also had the additional goal of assessing whether the safety of the formula could be improved by removing beta-carotene and reducing the amount of zinc to 25mg [71]. Results of the AREDS 2 trial will be discussed later with respect to the xanthophylls.

Studies assessing supplementation with antioxidant vitamins individually in AMD and without the use of other antioxidants have been less consistent. Low levels of vitamin C in the serum have been associated with an increased risk of AMD, however high

levels were not found to be protective [72]. Vitamin C is also a water soluble nutrient meaning reliable estimates of its status within the body are difficult to assess. Other studies have found no evidence for a beneficial role of vitamin C supplementation within AMD [73], [74], [75]. A recent randomised, double-masked, placebo-controlled trial which investigated 8 years of treatment and follow-up with 400IU/day of vitamin E (synthetic *α*-tocopherol) and 500mg/day of vitamin C (synthetic ascorbic acid) in 14,236 healthy male physicians, found a total of 193 incident cases of visually significant AMD. There were 96 cases in the vitamin E group and 97 in the placebo group (hazard ratio [HR], 1.03; 95% confidence interval [CI], 0.78-1.37). For vitamin C, there were 97 cases in the active group and 96 in the placebo group (HR, 0.99; 95% CI, 0.75-1.31), concluding that neither supplement had a beneficial effect on risk of incident diagnosis of AMD [73]. However, it is possible that the findings reflect the nutritional status of the study population as subjects were generally well-nourished and thus results may not apply to less well-nourished populations.

Similarly, supplementation of over 29,000 male smokers in Finland with 20 mg/day of beta-carotene and 50 mg/day of alpha-tocopherol for six years did not decrease the risk of AMD compared to placebo [76]. In the vitamin E cataract and ARM trial, (randomised controlled trial) 1204 men and women aged 55–80 years were randomly allocated vitamin E (335 mg of natural d-α tocopherol) or placebo and followed up for 4 years. 82% of the subjects had no incidence of AMD. It was found that the incidence of early AMD was 8.6% in those receiving vitamin E versus 8.1% in those on placebo. For late disease, the incidence was 0.8% versus 0.6%. This study indicated that daily supplement with vitamin E supplement did not prevent the development or progression of early or later stages of AMD [77]. A placebo-controlled trial in a cohort of 22,071 healthy US men found that beta-carotene supplementation (50 mg every other day) had no effect on the incidence of ARM- an early stage of AMD [78]. Moreover, two large systematic reviews of randomised controlled trials have concluded that there is no evidence that beta-carotene supplementation prevents or delays the onset of AMD [79], [80].

Xanthophylls

While the first AREDS study was in progress, evidence was emerging to suggest that the dietary xanthophylls L and Z may be more effective than other antioxidants nutrients in reducing AMD risk or progression due to their antioxidant and photo-protective properties [41]. Lutein and Z are part of the xanthophyll family of pigments, more specifically known as xanthophyll hydroxycarotenoids [81]. Within the eye,

these carotenoids are present in higher concentrations than other human tissues being particularly prominent in the peripheral retina, the iris and lens [82]. Lutein, Z and a related compound meso-zeaxanthin (MZ) are at their highest concentrations in the macula, were they are known as macular pigment (MP) [83]. The protective properties of the MP are now well established and include the ability to interact with free radicals, prevent lipid peroxidation and filter out high energy, damaging, blue light [84], [85], [86]. The macular pigment has biochemical significance to ocular health by possibly preventing disease onset and sustaining visual functionality through its antioxidant capabilities. Reactive oxygen species may be formed in the retina due to its high demand for oxygen. These highly oxidised radicals have shown to induce apoptosis of the macular photoreceptors; however L has been shown to scavenge the oxygen intermediate by quenching them via the numerous unconjugated double bonds in the L molecule [87]. Lutein has also been shown to inhibit lipid peroxidation by decreasing lipofuscin (lipid residue from liposomal digestion), which may contribute to drusen formation [88] [89] in cultured RPE cells [90]. By acting as a blue light filter, the lens and macular pigments can also protect the photoreceptor cells responsible for vision, from light induced damage. Lutein in particular has been shown to have the highest blue light filtering properties [84].

Lutein and Z are obtained by the human body exclusively from dietary sources [91], [81], and MZ is thought to be converted from L in the macula [92], [82]. Therefore, an individual's ocular tissue concentrations of these nutrients can vary depending on diet and lifestyle habits. It has been reported that around 78% of dietary L and Z is obtained from vegetables, with the highest concentrations found in dark green leafy vegetables, such as kale and spinach [93], [94]. Corn and corn products are confirmed as being a major source of Z [95]. Eggs also contain high levels of L and Z and have enhanced bioavailability of carotenoids in this form due to the fat which they contain [96]. Similarly, cooking, or ingesting other carotenoid-containing foods with dietary fat, or choosing a nutritional supplement that contains L and Z bound in oil, can also increase the bioavailability of L and Z [97]. It appears that humans intake low levels of MZ from the diet, however research is still on going in this area. Some food items have been reported as being rich sources of MZ such as eggs from hens fed MZ [114], and specific species of edible sea food such as shrimp.

Macular pigment optical density (MPOD – the amount of MP measured in the retina) can be increased with appropriate dietary modification: either by supplementation with L and Z or by consuming L and Z rich foods, as demonstrated in human and animal epidemiological and intervention trials [98-106]. A large body of this evidence

suggests that doses of 10mg/day or higher of L are associated with the most positive effects on MPOD [99-101,103,104]. One research group has investigated the relationship between supplemental MZ and MPOD. Their findings suggest that MPOD may also be increased with supplements which contain all three macular carotenoids, potentially offering advantages over preparations lacking in MZ. A study involving high MZ (20mg per day), and low L and Z supplementation reported that MPOD increased significantly over a period of 120 days. The average rate of increase for MP in both eyes of all 10 subjects was 0.56 mAU/day (range 0 to 2.2 mAU/day). The average rate of increase in MPOD was very similar to the value of 0.53 mAU/day observed in an earlier supplementation study where subjects took 20 mg/day of lutein. A milli absorbance unit (mAU) refers to the amount of light absorbed by a material, in this case the macula. At 20 mg/day, over a 120-day period, the predominantly MZ supplement was capable of increasing MPOD by an average of 18% [26].

Other factors which may influence MP levels include smoking habits (lower levels in smokers) [107], iris colour (lower levels with lighter iris colour) [108], gender (higher levels reported in men in some studies) [109], [110], and body fat (lower levels in those with a high percentage of adipose tissue) [111], [112]. The possible explanations for a relative lack of MP among cigarette smokers include a poor diet (with consequentially reduced levels of antioxidants) and/or increased overall oxidant load associated with tobacco. Smokers also frequently display evidence of inflammation, due to increases in the systemic inflammatory marker C-reactive protein [113] [114]. Inflammation in both smokers and non-smokers is inversely related to serum carotenoid concentrations [115]. Studies have also reported that elevated concentrations of C-reactive protein are an independent risk factor for AMD [116]. The inverse relationship between increased adipose tissue and MPOD may be explained by the competition between the retina and adipose tissue for the absorption and uptake of carotenoids, with the idea that adipose tissue acts as a sink and a reservoir for these nutrients [111], [117], [118]. This is because among all the tissues that store carotenoid pigments [119, 120] adipose tissue contains more than 80% of the body carotenoids [121]. Furthermore, increased body max index has been reported to result in increased oxidative stress [122], [123], [124], and reduced antioxidant defence mechanisms [125] which have been linked to the pathogenesis of AMD [126]. However, it is also possible that a relative lack of MP in obese subjects simply reflects a poor diet among those persons, as it has been demonstrated that obesity is correlated with reduced dietary intake of the carotenoids which comprise the macular pigment [119], [118].

Supplementation with L and Z has been correlated with a delay in progression or onset of AMD in some epidemiological and intervention studies, therefore on-going research into its role is important. The AREDS 2 is the largest trial to date that has investigated the effect of omega-3 fatty acids and/or L and Z within AMD. It was a multi-centre, randomised trial designed to assess the effects of oral supplementation of the macular xanthophylls (L and Z) and/or long-chain omega-3 fatty acids (DHA and EPA) on the progression to advanced AMD. Due to the possible side effects associated with high dose zinc and beta-carotene supplementation the trial also investigated whether a modified supplement with reduced zinc and/or no beta-carotene provided benefits similar to the original AREDS supplement. Inclusion criteria were males and females aged 50 to 85 years who were present with bilateral large drusen (≥ 125 microns) or large drusen in one eye and advanced AMD in the fellow eye. A study eye (eye without advanced AMD) may have been present with definite geographic atrophy not involving the centre of the macula without evidence of drusen, i.e. anybody with dry AMD in both eyes and dry AMD in one eye and wet AMD in the other eye [10]. In the primary analysis, participants were given one of four supplements to take on a daily basis, which included a placebo, L/Z, omega 3 fatty acids, or L/Z plus omega 3 fatty acids. Participants were also given one of four versions of the original AREDS formula to take simultaneously. The following figure shows a summarised study design key of the AREDS 2 trial.

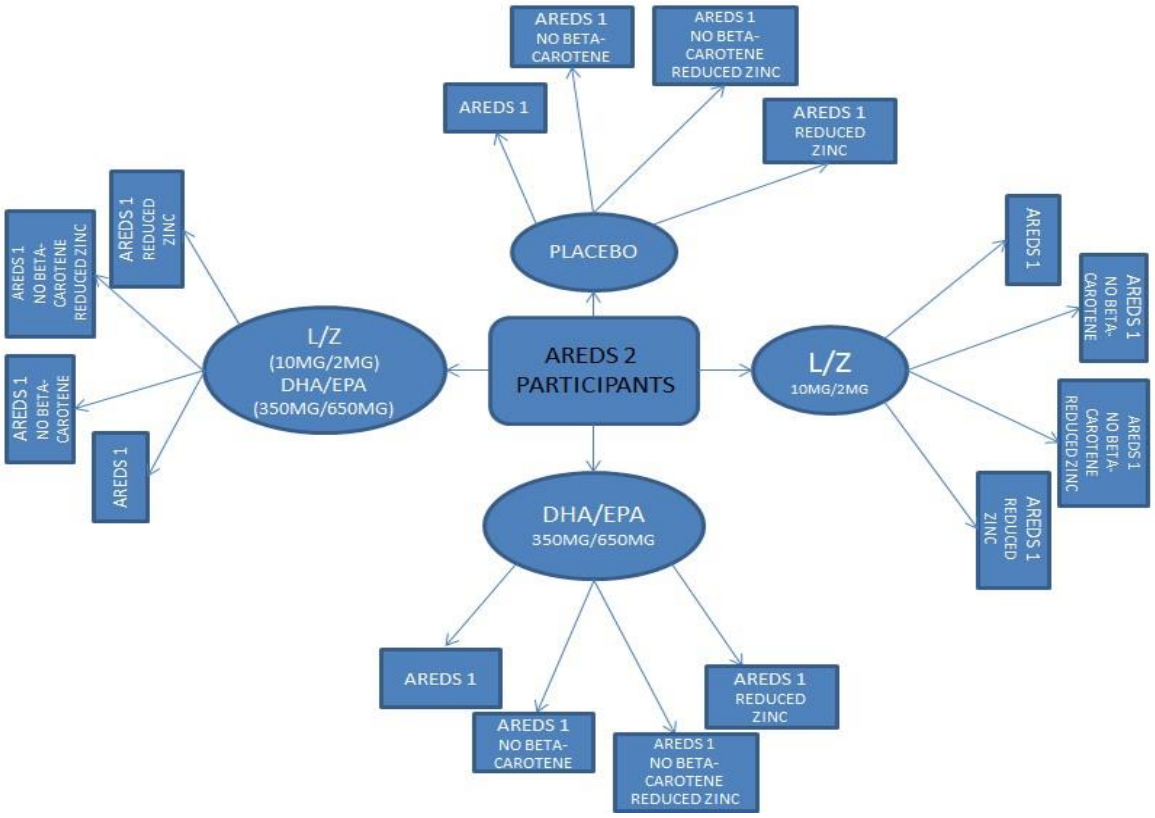

Figure 2.2: AREDS 2 study design key. L/Z – Lutein/zeaxanthin, DHA/EPA – docosahexaenoic acid/eicosapentaenoic acid (omega-3 long-chain polyunsaturated fatty acids), AREDS 2 – Age-related eye disease study 2.

The study found that overall there was no additional benefit of adding omega 3 fatty acids (DHA 350mg EPA 650mg), 10 mg of L and 2 mg of Z to the original AREDS formula of antioxidants. However, participants who took the AREDS formulation with no beta-carotene but with L and Z at these doses, had their risk of progression to advanced AMD reduced by 18% compared to those participants who took the AREDS formulation that contained beta-carotene without L and Z. In addition, participants who had a low dietary intake of L and Z at the start of the study (≤ 0.823 mg per day), but who took the AREDS formulation with L and Z during the study, were 25% less likely to develop advanced AMD compared with participants with similar dietary intake who did not take L and Z. There was no benefit from L and Z supplementation to those who consumed ≥ 1.030 mg of L and Z per day in their diet [71].

Investigators have suggested that beta-carotene may have masked the effects of L and Z in the overall analysis because it competes for absorption in the body. Participants who took beta-carotene and L and Z, had lower blood levels of both

compared with those who only took L and Z. The investigators also found that former smokers, who made up approximately half of the study population, were at greater risk of developing lung cancer if they took the formula which contained beta-carotene. This means it is likely to be safer for anybody who has ever smoked not to take supplements that contain beta-carotene. The results suggest that taking a combination of 500 mg vitamin C, 400 IU vitamin E, 25 mg zinc, 2 mg copper, 10 mg L and 2 mg Z may reduce the risk of progression to advanced AMD in people who already have signs of the disease. Including L and Z in the original AREDS formulation rather than beta-carotene appears to be safer and more effective. Reducing the amount of zinc from 80 mg to 25 mg does not appear to impact on the effectiveness of the formulation, and a formulation with a reduced zinc content is very likely to result in fewer gastrointestinal side effects [71].

Other large scale epidemiological studies such as the NHANES and the EDCC trials provide evidence that antioxidant status, particularly dietary xanthophyll intake may protect against the onset of AMD [127], [72]. In 2006, the CAREDS trial concluded that L-rich and Z-rich diets may protect against intermediate AMD in female patients less than 75 years of age [128]. The Blue Mountain Eye study reported that higher dietary L and Z intake reduced the risk of incident early or neovascular AMD over 5 and 10 years [129]. Moreover, a number of small randomised controlled trials have reported positive effects of lutein supplementation on visual performance in AMD patients, in terms of visual acuity, contrast sensitivity, glare recovery and visual distortion. Similarly, the majority of these trials have found improved measures of visual function in patients given 10 mg or higher per day with L alone or combined with Z and other antioxidants [130-133]. Supplementation with lower doses has not been found to show these results in some studies [134], [135]. Further studies are needed with more patients, of both genders, and for longer periods of time to assess long-term effects of L alone or L together with a broad spectrum of antioxidants on visual function. The AREDS 2 trial found no effect of L and Z together on visual acuity [71]. The trial analysed the progression to moderate or worse vision loss, defined as a reduction of 15 or more letters, as a secondary outcome. These results suggest that findings from smaller trials which suggest an inverse association between L and Z supplementation and visual performance should be treated with caution [130-133].

Omega-3 fatty acids

Omega-3 fatty acids are essential nutrients meaning humans have to obtain them exclusively from dietary sources. They include alpha-linolenic acid (a short-chain omega-3 fatty acid), docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), (both long chain omega 3 fatty acids). Alpha-linolenic acid is the dietary precursor to both DHA and EPA and is converted to a long-chain omega-3 fatty acid [136]. Omega-3 fatty acids are well known to exhibit anti-inflammatory, anti-atherosclerotic and anti-thrombotic effects on vascular tissue [137], [138] which have been largely correlated with a reduced risk of cardiovascular health problems in several epidemiological studies [139], [140]. It is hypothesized that cardiovascular disease and AMD share similar mechanisms and risk factors, as omega-3 fatty acids such as DHA have been found in high concentrations in the retina, where they are important structural components of vascular tissue and retinal photoreceptor- rod and cone outer segments, which are systematically shed and turned over during the visual cycle [141], [142].

Long-chain omega-3 fatty acids predominantly found in oily fish, may also protect against oxidative damage and help modulate retinal cell gene expression, cellular differentiation and cellular survival [143]. The critical role of DHA in normal retinal function is well documented in animals [144], [145], and humans [9], [146], [147]. It has an influence on cell membrane integrity and permeability, modulates the activity of enzymes and receptors on the membrane and acts as a precursor for the synthesis of other important biological molecules [9]. Observational studies suggest that omega-3 poly-unsaturated fatty acids (PUFA) are protective in the eye. A 2008 meta-analysis of epidemiological studies reported that a high intake of omega-3 fatty acids and fish intake at least twice a week may reduce the primary risk of both early and late AMD by up to 38% [148]. In a 5-year follow-up study of the Blue Mountains Eye Study cohort, fish consumption at least once a week was associated with a 40% reduction in incident early AMD and more frequent consumption of fish (3 times a week) was found to be protective against late age-related maculopathy (ARM) [149]. Other studies have found similar findings [150-153]. Some prospective studies also reveal a decreased likeliness of AMD progression in those with early AMD [154-156]. It is important to note that in these studies, positive correlations involving the risk or progression of AMD were only seen in subjects who had a low dietary intake of omega-6 fatty acids. These findings support similar evidence found in other cohort studies [157], [154] highlighting the importance of maintaining a healthy ratio between these two essential fatty acids in the diet [158, 159]. Increased dietary intake of omega-6 fatty acids can lead to oxidation of LDL cholesterol, platelet aggregation and

interference with the incorporation of EPA and DHA in cell membrane phospholipids [158]. Western diets are characterized by high omega-6 and low omega-3 fatty acid intake, with a ratio of omega-6 to omega-3 of 10–20:1 [17] [18], which is due to the increased consumption of omega 6-rich vegetable oils, and the low consumption of fish [19]. Optimal dietary intakes of the n-6 : n-3 ratio are however estimated to be around 1–4 : 1 [160].

There is a small amount of conflicting evidence which suggests no effect on the risk or progression of AMD [161], [71] or an increased risk of progression of AMD with omega-3 fatty acids [162], [163]. Primary, secondary and subgroup analyses from the AREDS 2 trial demonstrated no additional benefit on the reduced risk of progression to advanced AMD by adding the omega-3 fatty acids EPA and DHA to the original formulation. The doses used (DHA 650mg and EPA 350mg) were also found to exhibit no adverse effects after 5 years of follow up. These null results may mean that omega- 3 is simply an ineffective treatment option for AMD or it could be related to an inadequate dose, inadequate duration of treatment, or both [71].

Omega-3 supplements containing the doses used in the AREDS 2 trial may not be recommended for AMD patients; however, it is still essential that adequate amounts are met through diet alone as they have important cardiovascular health benefits. The UK Department of Health recommends consuming at least 1-2 portions of oily fish per week [164].

Zinc

Zinc is the second most abundant trace mineral in the body as it is an essential component of over two hundred enzymes. It plays an important role in a number of physiological processes including immunity, reproduction, and neuronal development [165]. In the eye it acts as a cofactor for the antioxidant enzymes retinal dehydrogenase and catalase and is also involved in retinal metabolism [166]. Low levels of zinc are thought to play a role in the development of AMD for several reasons. Firstly, zinc is highly concentrated in the RPE [166], [167], [168] and retinal zinc levels have been shown to decline with age [169], [170], [171]. Thus, it is hypothesized that zinc supplementation may aid retinal health.

The elderly are at particular risk from zinc deficiency [169], [170], [171], [172], which can lead to a reduction in T lymphocytes and B lymphocytes and the macrophage through increased apoptosis. Zinc deficiency can also alter the function of alcohol dehydrogenase in the retina [173], which can result in increased overall vitamin A

uptake. The possible accumulation of retinyl esters in the RPE may interfere with normal biosynthesis and could also produce a toxic effect. Furthermore, zinc deficiency promotes lipid peroxidation and damage to lipid membranes. [174]

The evidence which suggests a link between dietary zinc and AMD comes mostly from the AREDS 1 and 2 trials. There have been no randomised or case controlled investigations involving zinc and the risk of AMD; however other prospective data has demonstrated some positive results. A small number of cohort studies assessing dietary intakes of zinc in elderly subjects have found an inverse relationship between high zinc intake and the incidence of early AMD [175], [150] and any stage of AMD [176]. One study also reported a decrease in pigment abnormalities in those with a high consumption of zinc intake from food or supplements [75].

In terms of zinc intake and the progression of AMD, a large proportion of the perceived benefit comes from the AREDS 1 trial which found a 25% reduction in AMD progression in patients with early AMD using 80mg of zinc plus other antioxidants daily. Two smaller sized randomised clinical trials have also found that supplementation with zinc alone improves visual function in patients with AMD. The doses used in the studies were 25mg [177] and 100mg daily [178]. There were little adverse associations in the subject group who received 25mg of zinc. Other large cross sectional studies involving zinc and the progression of AMD have yielded conflicting results [7], [179], [180].

The negative effects of high zinc supplementation are not fully understood. It is suggested that intakes above the daily recommended values are associated with copper deficiency, [180], [181]. It was also found in the AREDS 1 trial that a small number of subjects (7.5%) receiving 80mg per day of zinc suffered with genitourinary infections [8]. The AREDS 2 trial found that reducing the amount of zinc from 80 mg to 25 mg does not appear to impact on the effectiveness of the formulation. Rates of reported gastrointestinal disorders and hospitalisations for genitourinary diseases were similar in the 2 randomly assigned groups (high-dose zinc, low-dose zinc) in the trial, however recommending an AREDS 2 formulation with a reduced zinc content (25mg) is considered safer [71].

B vitamins

The B vitamins are a group of water soluble nutrients. These vitamins, often referred to as B complex vitamins, play a role in energy conversion which help the body

metabolise fats and protein. B complex vitamins are needed for a healthy liver, skin, hair, and eyes. They also help with the function of the nervous system. Vitamin B2 (riboflavin) acts as an antioxidant, has a role in the synthesis of steroids and erythrocytes (red blood cells), and in maintaining the integrity of mucous membranes. Vitamin B6 (pyroxidine) refers to a group of nitrogen-containing compounds with three primary forms: pyridoxine, pyridoxal and pyridoxamine. Vitamin B6 participates in over 100 enzymatic reactions and has a reported role in gluconeogenesis (the synthesis of glucose from non-carbohydrate sources) [182], lipid metabolism [183], erythrocyte metabolism [184], and immune function [185], [186]. Vitamin B12 is an essential cofactor for two enzymes: methylmalonyl-CoA mutase, is needed for fatty acid metabolism, and methionine synthase [187], which controls nucleic acid synthesis and methylation reactions within the body. Deficiency leads to megaloblastic anaemia via a reduced production of red blood cells.

Vitamins B6, B12 and folic acid regulate levels of a protein known as homocysteine in the blood. High levels of homocysteine are a significant risk factor for cardiovascular disease and research suggests that cardiovascular disease and AMD share a similar risk profile. Furthermore, high levels of homocysteine have been found in AMD patients, suggesting an association between elevated serum homocysteine levels and the risk of AMD [188-191]. Treatment with folic acid, vitamin B6 and vitamin B12 has been shown to reduce homocysteine levels. In a meta-analysis of randomised controlled trials, folic acid lowered plasma homocysteine levels by 25%, and an addition of B12 lowered levels by a further 7% [192]. Further evidence from controlled trials since this review which examine the effect of therapy to lower homocysteine levels within AMD risk or progression are limited. There is one relatively large scale randomised controlled trial which suggests that a combination of folic acid, vitamin B12 and B6 may reduce the risk of AMD in those with pre-existing cardiovascular disease. [193]

Although there are a significant proportion of studies suggesting an association between elevated plasma homocysteine and AMD regardless of the subtype, further evidence from controlled trials which examine the effect of these nutrients on homocysteine levels in relation to AMD risk or progression is limited and therefore they cannot be recommended as supplements. AMD patients can however try to include more foods in the diet which contain these nutrients such as whole grains, dairy products, leafy green vegetables, nuts, fish, beef, liver, poultry, potatoes and non-citrus fruits [194, 195].

1.3 Bioavailability of lutein and zeaxanthin within food sources

Lutein and Z are the only carotenoids found at appreciable levels in the macula [49, 62–66], where they are implicated in the maintenance of retinal health and possible prevention of the onset or progression of AMD. Lutein and Z have been shown to protect against reactive oxygen species [87], and to inhibit lipid peroxidation by decreasing lipofuscin (lipid residue from liposomal digestion), which may contribute to drusen formation [88] [89] in cultured RPE cells [90]. Lutein has also been shown to have the highest blue light filtering properties. The concentration of L and Z in the macula and their potential biologic function may be modified by diet or supplement use and they are therefore the central focus of AMD research regarding nutrition [98, 99].

Kale (*Brassica oleracea var. sabellica*), which is commonly cultivated in central and northern Europe and North America, belongs to the Brassicaceae family of vegetables. It is an excellent source of dietary carotenoids and typically has the highest concentrations of L and Z amongst other green leafy vegetables [196], [94, 95]. However, the qualitative and quantitative composition of carotenoids in fruits and vegetables is known to vary with multiple factors such as, cultivar or variety, maturity at harvest, climate, farming practice and post-harvest processing and storage [197-200]. Due to the high concentration of L and Z in dark green leafy vegetables their consumption is recommended in order to increase MP as prevention of onset or progression of AMD [55, 201]. It is therefore important to establish the most effective way to use these important food sources such as kale, in order to preserve the levels of L and Z. Results from such a study will provide patients and clinicians with a clearer understanding of the effects of pre-and post-harvest conditions on the carotenoid content of vegetables which will enable precise suggestions for increasing retinal levels of these nutrients. Firstly, it is important to identify the specific effects of pre- and post-harvest conditions on L and Z concentrations in kale from the current literature, and thus to identify where further research is needed.

A literature review was carried out to determine the compositional carotenoid variations in dark green leafy vegetables due to variety/cultivar, stage of maturity, climate or season, farming practice, storage, and processing effects. The review can be found published here: **Walsh, R. Bartlett, H. Eperjesi, F. Variation in Carotenoid Content of Kale and Other Vegetables: A Review of Pre- and Post-Harvest**

Effects, *J. Agric. Food Chem.*, 2015, 63 (44), pp 9677–9682. The abstract is presented below and the full paper is attached in the appendix (appendix 1).

Abstract

Lutein and zeaxanthin are carotenoids that are selectively taken up into the macula of the eye, where they are thought to protect against the development of age-related macular degeneration. They are obtained from dietary sources, with the highest concentrations found in dark green leafy vegetables, such as kale and spinach. In this review, compositional variations due to variety/cultivar, stage of maturity, climate or season, farming practice, storage, and processing effects are highlighted. Only data from studies which report on lutein and zeaxanthin content in foods are reported. The main focus is kale; however, other predominantly xanthophyll containing vegetables such as spinach and broccoli are included. A small amount of data about exotic fruits is also referenced for comparison. The qualitative and quantitative composition of carotenoids in fruits and vegetables is known to vary with multiple factors. In kale, lutein and zeaxanthin levels are affected by pre-harvest effects such as maturity, climate, and farming practice. Further research is needed to determine the post-harvest processing and storage effects of lutein and zeaxanthin in kale; this will enable precise suggestions for increasing retinal levels of these nutrients.

Author	Cultivar	Maturity	Climate	Farming Practise
Mercadante et al 1991 [202]	L concentrations in two field grown cultivars in Brazil ranged between 11.4mg/100mg of fresh weight (‘tronchuda’) and 7.1 mg/100mg of fresh weight (‘manteiga’).		Carotenoid concentrations were found to be higher in the winter than the summer for kale harvested in open fields, suggesting that sunlight and high temperature may promote photo degradation when vegetables are not protected by roofing or packaging	The study compared kale of the same cultivar at the same stage of maturity produced on these opposing farms, and found significantly higher concentrations of all carotenoids in samples collected from the natural farm
Kopsell et al 2004 [198]	L concentrations ranged from 13.43 mg/100g fresh weight to a low of 4.84 mg/100g in cultivars.			
De-Azevedo, et al. 2005 [199]		L and beta-carotene concentrations were much higher in fully expanded mature kale than younger leaves	‘Manteiga’ kale purchased from a Brazillian supermarket and cultivated under polythene roofing, had a higher concentration of all four principal carotenoids in the summer as opposed to winter	
Lefrsrud, et al 2007 [203]		Mature fully expanded kale leaves, harvested between 1-3 weeks accumulated higher carotenoid concentrations than young leaves		

Table 2.2: Summary of studies which have investigated the pre-harvest effects on carotenoid concentrations (those which include L) in kale.

Author	Storage	Boiling/Blanching	Steaming	Frying	Microwaving
De-Azevedo, et al. 2005 [199]	In minimally processed kale monitored during 5 days of storage at 7–9 ◦C, β-carotene, L, violaxanthin and neoxanthin were reduced by 14, 27, 20 and 31% respectively.				
Murador et al. 2016 [204]		Boiling after 4 min showed a higher degradation of total carotenoids in kale relative to the raw sample (77%; $p < 0.0001$). Raw kale contained 56.18 ± 2.9 µg/g of lutein, after boiling this was 6.9 ± 0.54 µg/g.	Steaming kale for 5 min showed a degradation of 72% ($p < 0.0001$) of total carotenoids relative to the raw sample, however lutein levels decreased further than boiling to 5.81 ± 1.14 µg/g.	Stir-fried kale (4 min with soybean oil) showed the best conservation of total carotenoids with a reduction of 55% ($p < 0.0001$) of the total carotenoids. For lutein, however, levels decreased further than all other cooking methods to 4.66 ± 1.81 µg/g.	

Table 2.3: Summary of studies which have investigated the post-harvest effects on carotenoid concentrations (those which include L) of kale.

The above tables summarise the findings of a small number of studies which have investigated the pre-and post-harvest effects on the carotenoid concentrations in kale. These include studies which have investigated the variation in L concentrations for different kale cultigens and the effects of maturity, climate, farming practice, storage and processing on kale carotenoid concentrations. One study to date has investigated the post-harvest storage effects on the carotenoid concentrations, including L, in kale. Since publication of the literature review, only one study to date has investigated the post-harvest domestic cooking effects on the L concentrations in kale.

1.4 Summary

Overall, there is a great deal of potential for the benefits of including nutrients such as antioxidant vitamins, L and Z, omega 3, and B vitamins in the diet of AMD patients and it is therefore important that people aged between 50 and 85 years who present with early signs of the disease are counselled about appropriate dietary modification or supplementation. The AREDS 1 and AREDS 2 are the largest clinical trials that have taken place in the field of nutrition and ocular disease and it is likely that eye care practitioners would feel more comfortable about recommending this specific combination of nutrients if dietary supplementation is chosen based on an individual's suitability to the study criteria. Results from the AREDS 2 trial suggest that taking a combination of 500 mg vitamin C, 400 IU vitamin E, 25 mg zinc, 2 mg copper, 10 mg L and 2 mg Z may reduce the risk of progression to advanced AMD in people who already present with signs of the disease. In this new AREDS formula beta-carotene has been replaced with L and Z, and the high levels of zinc have been reduced. In terms of dietary modification, patients should be encouraged to consume more dark green leafy vegetables such as kale and spinach to increase L levels in the diet. Oily fish should also be recommended in moderation to increase essential fatty acids in the diet as well as other food sources containing antioxidant vitamins and minerals such as nuts, seeds, and other fruits and vegetables. To provide patients with unsurpassed advice on enhancing MP levels through increased consumption of dark green leafy vegetables such as kale, it is essential that we gain further knowledge on how these food sources should be cooked and stored in order to best preserve the levels of L and Z.

This chapter has reviewed the information regarding nutrition and AMD. In chapter two, an investigation of dietary intake amongst people with AMD will be described.

Chapter 2: Dietary analysis in AMD patients by repeat 24 hour recalls

2.1 Background and rationale

Age-related macular disease (ARMD) is a multifactorial degenerative condition affecting the central area of the retina. While treatments for neovascular AMD (wet AMD) have been used to limit the progression of the condition and reduce the risk of vision loss, proven treatments for the atrophic (dry) form of AMD are yet to be established. Preventive interventions through dietary modification and supplementation have therefore been the focus of a number of observational and randomised clinical trials, [4, 6-10]. This is in part due to the fact that they are more affordable than clinical therapies and do not require specialists for administration. In addition, many studies suggest that different nutritional factors, such as antioxidants and lipids, are believed to influence the development/progression of AMD with few, if any, adverse effects. The nutrients that have been associated with the prevention of onset or progression of such condition include the antioxidant vitamins C, E and the vitamin A precursor beta-carotene, the xanthophylls lutein (L) and zeaxanthin (Z), omega-3 fatty acids DHA and EPA, zinc, and some B vitamins (folic acid, B2, B3, B6, and B12) [8, 10, 55, 189, 193, 205].

This has been broadly supported by the AREDS 1 [8] and AREDS 2 [10], the two largest randomised clinical controlled trials in the field of nutrition and AMD. The first AREDS study documented that taking a supplement containing vitamins E and C, beta-carotene and zinc reduced the risk of progression of the disease by 25% in those with intermediate or advanced stages of the disease in one eye. Since then, the carotenoids L and Z have been identified as nutrients that can provide a protective role in the progression of AMD due to their antioxidant and photo-protective properties [50]. Collectively, L and Z form the macular pigment [206] which interacts with reactive oxygen species, prevents lipid peroxidation and filters out high energy blue light. The follow up AREDS 2 trial was the largest trial to date which has investigated the effect of omega-3 fatty acids and/or L and Z within AMD. The study found that overall there was no additional benefit of adding omega 3 fatty acids (DHA 350mg EPA 650mg), 10 mg of L and 2 mg of Z to the original AREDS formula of antioxidants. However, participants who took the AREDS formulation with no beta-carotene but with L and Z at these doses, had their risk of progression to advanced AMD reduced by 18% compared to those participants who took the AREDS formulation that contained beta-carotene without L and Z [10].

Not all AMD patients will benefit from an AREDS supplement. Those with very early stages of the disease may wish to consider dietary modification, as increasing intake of L and Z has been shown to increase macular pigment levels [207]. Macular pigments such as L and Z have biochemical significance to ocular health by possibly averting AMD onset and sustaining visual functionality through their potent antioxidant capabilities [208], [103]. Nutrients such as L and Z are not formed within the body and so can only be obtained from our diet. Patients are presented with a wealth of nutritional information available from a variety of sources such as magazines, newspapers and the internet. Conflicting information, a lack of evidential research and aggressive marketing campaigns have led to confusion among patients and practitioners in what supplements to take, and what foods should be consumed in order to maximise absorption of L and Z [209]. Previous work from the author that characterised AMD patients seeking the services of the Macular Society (A UK charity devoted to helping those with diseases of the macula) and determined their awareness of the relationship between nutrition and AMD, found that over half (63%) of the participants felt that they did not have enough information on lifestyle factors and their relationship to AMD [210]. Following the results of the AREDS2 trial, the Macular Society (MS) have advocated the use of the AREDS2 formulation, where appropriate, and eating vegetables that are L and Z rich. They also encourage the intake of a wide variety of fruits and vegetables in the diet to ensure an adequate supply of antioxidants such as vitamins A, C, E and zinc. Patients have access to a variety of nutritional information resources including leaflets, magazines and a patient portal displayed on the main website.

One recent study within MS members analysed whether this 'informed' population were following the nutritional guidelines for AMD by analysing the individual dietary intake of nutrients and comparing the results with people not affected by AMD [211]. Interestingly, after assessment via a single 24 hour recall, it was found that many AMD participants were under-consuming nutrients considered to be useful for their condition, despite the wealth of nutritional information contained on the MS website. On average AMD patients consumed only 1.6 mg of lutein per day. These findings correspond with another study which estimated the intake of antioxidant nutrients in wet AMD patients living in the Balearic Islands, Spain using 24 hour recalls and food frequency questionnaires [212]. This study reported that the majority of patients showed inadequate antioxidant nutrient intake (<2/3 of Recommended Dietary Intake, (RDI)), and more than 60% of patients showed a deficient intake (< 1/3 recommended 10mg) of L and Z. The fat and saturated fatty acids (SFA) intake of study participants

were also higher than recommendations; 61.9% of men and 58.1% of women were overweight; and 83% of patients (90.5% men and 77.4% women) showed body fat mass over the cut-off limits. It was concluded that the food pattern of wet AMD patients should be improved by means of an increase in the consumption of antioxidant rich foods, and a decrease in SFA rich foods [212].

In these previous studies, dietary intake data was collected via a single 24-hour recall. The 24-hour recall method is a popular tool for investigating the relationship between diet and disease. This is mainly due to its high response rate and its ability to obtain detailed information, and also because recall of intake over a longer time period is problematic particularly in the older generation due to the limitations of memory [213]. However, a single 24-hour recall is not considered to be representative of habitual diet at an individual level but more suited to analysing group variations as just a snap shot of dietary intake [214]. If the distribution of usual individual food intakes within the groups is also needed, at least two non-consecutive days of intake per individual are required to evaluate day-to-day variability. Therefore repeat 24 hour recalls are becoming more popular to asses a typical diet at an individual level [215, 216].

The aim of this study was to follow up a previous dietary assessment study and investigate dietary intake in AMD patients with the more robust method of a repeat 24-hour recall. The nutrient intake of a smaller group of AMD subjects from the MS was analysed using this method over three non-consecutive days.

2.2 Methods

Ethics

This study was approved by the Aston University Ethics Committee (Ethics application 728). Verbal informed consent was obtained from all subjects and formally recorded.

Sample size

To calculate an appropriate sample size, the author referred to the research group's previous work on single food recall dietary analysis [211], in which participants without AMD consumed an average of 647 kilocalories more than those with AMD, and in which AMD patients consumed on average 485 kilocalories fewer than the DRV. In order to detect a difference between groups of 300 kilocalories, a sample size of 40 was required. The sample size of 40 participants was calculated to observe a medium effect size (d=0.5), with 80% power and a type I error probability of 5%.

Recruitment of subjects

People who called the MS helpline between February 2015 and July 2015 were invited by staff to take part in the dietary assessment study. A script was given to all helpline staff to help with the recruitment process. It explained the study procedure and protocol including the inclusion and exclusion criteria for those wishing to be involved. The only pre-requisites were that participants should be aged over 50 and have been diagnosed with a form of AMD; the exclusion criteria were the inability to hear and reply to questions in English over the telephone.

Method of dietary assessment

When a helpline caller agreed to take part in the study, their details (name and contact number) were passed to the investigator, who then called them back at a convenient time to conduct each dietary assessment. Three dietary assessments were completed for each participant by a 24-hour recall from two non-consecutive week days and one weekend day. Background information about the study and a consent statement were read out to each participant at the start of the follow-up call, before the first dietary recall was carried out. Participants were asked to confirm that they had been diagnosed with AMD. The participants were given the opportunity to ask questions about the study both at recruitment and when they were first called by the investigator. Each dietary recall included a description of everything that was eaten or drunk in the previous 24 hours. A questionnaire for data collection purposes before the recall was also asked of the participant and included questions such as:

- Age/Gender
- Do you take any vitamin or nutritional supplements for your eyes?
- How long have you suffered with AMD?
- Are you registered severely sight impaired or sight impaired?
- How would you describe your vision now?
- Do you take any nutritional supplements?
- Do you smoke?

The short questionnaire did not include any questions that might cause concern for the participant. Participants had the opportunity to ask questions about nutrition for eye health that may have been prompted by the dietary assessment at the end of the interview and delivered a standard set of nutritional information about diet and AMD to each. All participants were given a contact number to call if they thought of any questions after the interview had ended.

Summary of telephone interview protocol

- Introduction and confirmation that the participant was willing to participate
- Arrangement of an alternative interview time if required
- Withdrawal from the study if required
- Study information read out to the participant
- Consent statements read out to the participant and consent confirmed
- Questionnaire of data collection delivered and responses recorded
- Dietary assessment interview and responses recorded
- Participant given the opportunity to ask questions
- Participant provided with contact numbers in case any questions arose later
- Telephone interview ended

Method of dietary analysis

Dietary data was collected from a total of 45 participants. The conversion of food consumption data to nutrient intakes was completed via the food database software 'Al La Calc' (Red Hot Rails LLP, Doncaster UK) where each participant's daily food intake is analysed using the USDA (United States Department of Agriculture) SR25 food database (http://ndb.nal.usda.gov/). Each participant was registered in to the software anonymously and 24-hour recall data was added in to separate one day 'recipes'. Averages for individual nutrients were accounted for over the three recalls, leaving one total figure for each nutrient. A table was created in excel displaying the participant number with subsequent columns containing information such as - Age, gender, AMD type, number of eyes affected by AMD, duration of AMD, perceived vision, visual registration, supplement status, smoking status, total energy, total fat, of which saturates, carbohydrates, of which sugars, fibre, protein, potassium, calcium, magnesium, iron, zinc, selenium, vitamin D, vitamin E, vitamin b6, vitamin b12, folate, vitamin C, vitamin A and L/Z combined. Averages and standard deviation for each nutrient were collected from the total number of participants. Data was then analysed in statistical software IBM SPSS version 20 (IBM UK Ltd, Portsmouth, Hampshire) to draw comparisons between results using parametric (independent t-test, one-way ANOVA, Pearson product-moment correlation) and non-parametric tests (Mann Whitney U, Kruskal-Wallis, Spearmans rank-order) as not all the data was normally distributed. Normality of data was evaluated using a Shapiro-Wilk Test.

2.3 Results

Table 2.4 displays the demographic differences of the whole group of AMD subjects. In total, there were forty-five AMD participants aged 55-88 (mean 77 ± sd 8 years). Of the cohort, 27% were male and 73% were female. Dry AMD was more predominant within the cohort with 73% of subjects diagnosed with this form, compared with only 15% of subjects diagnosed with wet AMD. A small number of patients had both wet and dry AMD. The mean duration of diagnosis with AMD was 58 months. The majority of participants were not registered blind or partially sighted (81%), despite 30% of participants claiming that they felt their vision was 'poor' or 'very poor' on the day of the survey and 65% of participants had AMD in both eyes. Only 8% of participants felt that their vision was 'very good' and 15% felt it was 'good' on the day of the survey. The majority of patients took some form of dietary supplement on a daily basis (72%). Finally, only 8% of the cohort smoked – one important risk factor for AMD.

Characteristic	Characteristic	Percentage of AMD patients
Gender	Male	27%
	Female	73%
Perceived Vision	Very poor	4%
	Poor	26%
	Average	47%
	Good	15%
	Very good	8%
Visual Register	Blind	4%
	Partially sighted	15%
	None	81%
Supplements	Yes	72%
	No	28%
Smoke	Yes	8%
	No	92%
AMD type	Wet	15%
	Dry	73%
	One wet one dry	12%
Eyes affected	One eye	35%
	Both eyes	65%

Table 2.4: Selected demographic differences of AMD patients.

Tables 2.5 and 2.6 display results of the three-day dietary recall for subjects. Particularly important nutrients for AMD were included in the analysis and will be of focus such as L/Z. Table 2.5 shows the mean consumption and standard deviations of nutrients for the whole cohort as well as the range across the three days. Table 2.6

displays the mean consumption of various nutrients for males and females within the cohort together with the dietary reference values (DRV) for each constituent, as recommended by the UK government for those aged over 50 years [217]. The DRVs can be divided into three types: RNI - Reference Nutrient Intake (95% of the population's requirement is met), EAR - Estimated Average Requirement (50% of the population's requirement is met), LRNI - Lower Recommended Nutritional Intake (5% of the population's requirement is met). RNI's are used for protein, vitamins and minerals and vary by age. The DRVs for food Energy are defined as EAR's and have recently been updated by The Scientific Advisory Committee on Nutrition (SACN) for specific population groups. For those aged 50 years and above the new DRVs for daily calorie intake are as follows [218]:

	Unit	Mean whole Group	SD whole group
Energy	kcal	1594	249
Fat	g	58.2	15.4
of which saturates	g	21.7	7.3
Carbohydrate	g	202.7	41.6
of which sugars	g	85.2	25.5
Fibre	g	15.9	4.5
Protein	g	71.5	19.4
Sodium	mg	2318.4	746.3
Potassium	mg	2942.5	858.9
Calcium	mg	740.7	193.7
Magnesium	mg	284.8	72.0
Iron	mg	12.7	4.3
Zinc	mg	8.2	1.8
Selenium	µg	50.3	22.1
Beta Carotene	µg	2800.5	1912.8
Vitamin D	IU	35.7	60.9
Vitamin E	mg	7.2	2.6
Vitamin B6	mg	2.0	0.6
Vitamin B12	µg	7.8	10.4
Folate	µg	287.2	88.5
Vitamin C	mg	121.3	64.9
Vitamin A	IU	2150.3	2033.1
L/Z	µg	1691.7	1726.3
L/Z	mg	1.7	1.7

Table 2.5: Mean consumption, standard deviation and range across three days for the whole cohort of AMD patients. Please note: kcal refers to kilocalories, mg refers to milligrams, µg refers to micrograms, IU refers to international units and g refers to grams.

	Unit	Mean female (95% CI)	Mean male (95% CI)	DRV f >50	DRV m >50
Energy	kcal	1576 (1498-1654)	1644 (1577-1709)	1840-2079	2294-2581
Fat	g	56.7 (52.2-61.2)	62.5 (57.5-67.5)	70	95
of which saturates	g	22.2 (19.9-24.5)	20.4 (18.5-22.3)	20	30
Carbohydrate	g	205.4 (192.5-218.3)	195.4 (183.8- 207)	230	300
of which sugars	g	87.1 (79.7-94.5)	80.3 (71.9- 88.7)	90	120
Fibre	g	16.4 (15.0-17.8)	14.4 (13.2-15.6)	24	24
Protein	g	70.7 (64.3-77.1)	73.6 (69.5-77.7)	53	53
Sodium	mg	2201.3 (1985-2417.6)	2640.4 (2414-2866.5)	1600	1600
Potassium	mg	2969.3 (2696.6-3242)	2868.8 (2647.1-3091)	3500	3500
Calcium	mg	765.0 (710.8-819.2)	673.7 (607.4-740)	700	700
Magnesium	mg	287.2 (264.6-309.8)	278.3 (258.7-297.9)	300	270
Iron	mg	12.1 (10.9-13.3)	14.3 (12.9-15.7)	8.7	8.7
Zinc	mg	7.9 (7.4-8.4)	9.0 (8.4-9.6)	7	9.5
Selenium	µg	48.2 (47.7-48.7)	56.1 (48.1-64.1)	60	75
Beta Carotene	µg	3086.3 (2494-3678.6)	2014.5 (1575.5-2507)	-	-
Vitamin D	IU	37.9 (18.8-57.0)	29.6 (13.2-46.0)	400	400
Vitamin E	mg	7.2 (6.5-8.0)	7.4 (6.6-8.4)	10	10
Vitamin B6	mg	2.0 (1.8-2.2)	1.9 (1.7-2.1)	1.2	1.4
Vitamin B12	µg	7.7 (4.6-9.2)	8.1 (6.3-9.9)	1.5	1.5
Folate	µg	286.4 (260.7-312.1)	289.5 (259.3-319.7)	200	200
Vitamin C	mg	124.3 (104.3-144.3)	113.2 (94.5-131.9)	40	40
Vitamin A	IU	2493.1 (1839.1-3147)	1207.7 (608.5-1807)	2000	2333
L/Z	µg	1958.3 (1398.6-2517)	958.5 (654.3-1263)		
L/Z	mg	2.0 (1.4-2.5)	1.0 (0.7-1.3)	10	10

Table 2.6: Mean consumption of various nutrients for males and females (with 95% CI's), and the corresponding DRVs for those over 50 years old. Spaces indicate that there are no DRVs for that nutrient.

Results demonstrate that both males and females of all age groups failed to meet the government DRV for energy requirements (kcal), carbohydrates, fibre, selenium, potassium, vitamin D, vitamin E, and L/Z. For males, there were dietary concerns regarding vitamin A intakes. Both men and women's daily sodium intake was much higher than recommended. Of importance to AMD are the nutrients L/Z, beta-carotene, vitamin C, vitamin E and zinc. In comparison with the DRVs male and female subjects were under consuming L/Z and Vitamin E. Zinc was slightly below the DRV for male subjects. Dietary intakes of vitamin C in both males and females were substantially above the DRV.

		DRVs kcal	Mean kcal intake	% below DRV	Statistical Test (Mann- Whitney U, independent t test)
55-64 years	Male	2581	n=0	N/A	N/A
	Female	2079	1592 (n=3)	23%	z = -2.087 p= 0.037
65-74 years	Male	2342	1612 (n=4)	31%	z = -2.460 p = 0.014
	Female	1912	1678 (n=8)	12%	z = -1.795 p = 0.073
75+ years	Male	2294	1660 (n=8)	28%	z = -3.590 p < 0.001
	Female	1840	1523 (n= 22)	17%	t = -5.837 p < 0.001

Table 2.7: DRVs for calories in males and females of different age groups, with the corresponding mean calorie intakes of this cohort:

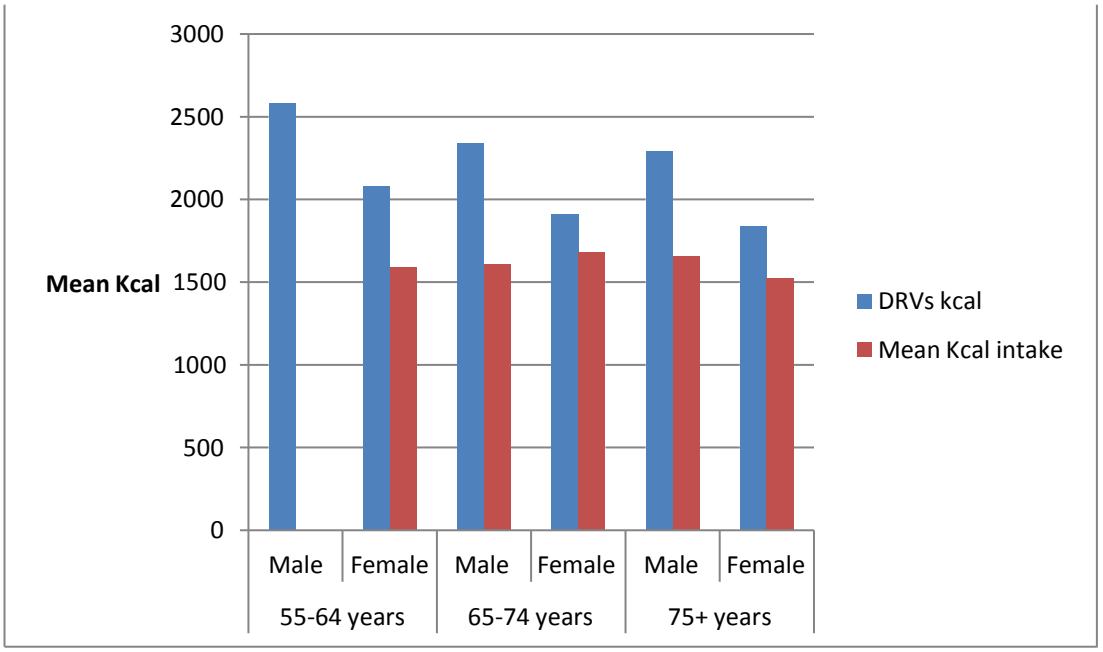

Figure 2.3: DRVs for calories in males and females of different age groups compared with the mean calorie intakes for males and females of this cohort.

Table 2.7 and figure 2.3 display the mean intake for calories of males and females in different age groups in comparison with the DRVs. Mean calorie intakes for both males and females of all age groups were consistently below the DRVs, with a substantial decrease ranging between 12-31% (Table 4). Results show that calorie intakes were significantly below the DRV for all males in the cohort (Man Whitney U test $p < 0.005$) and for females 55-64 years old (Man Whitney U test $p = 0.037$) and above 75 years old (independent t test $p < 0.001$). Females between the ages 65 and

74 years consumed an average of 1678 kcal per day, which was 12% below the DRV and not found to be a significant decrease (independent t test p > 0.005).

	Repeat 24-hour recall	24-hour recall	Statistical Analysis (independent t test)
L/Z (mg per day)	1.6	1.7	P=0.919
Calories (kcal per day)	1511	1594	P=0.254

Table 2.8: Comparison of L/Z and calorie intakes with data from a previous 24-hour recall study

Average L/Z and calorie intakes from this cohort were statistically compared with intakes from AMD patients in the previous 24 hour recall study carried out by our research group [211]. Results demonstrated that mean L/Z and calorie intakes were similar in both studies and were therefore not significantly different. No significant differences were found between L/Z or calorie intakes and patient demographics. Carbohydrate intakes were found to be significantly lower ($t(43) = 2.164$, $p = 0.036$) in those with AMD in both eyes ($M = 193.1$g, $SD = 41.64$) compared to those in one eye ($M = 220.1$g $SD = 36.76$).

2.4 Discussion

Comparison of L/Z and Calorie Intakes between Single and Repeated Dietary Recall:

Average L/Z and calorie intakes from this cohort of AMD patients were almost identical to the previous study from our research group which investigated a similar group of AMD patients using a 24 hour recall dietary assessment method [211]. Results demonstrated that there was no significant difference between L/Z intakes (independent t test $F = 197$ $p = 0.919$) and calorie intakes (independent t test $F = 197$ $p = 0.254$) in the two studies. These nutrients were found to be considerably lower than the recommended amounts in both studies. Although a smaller number of subjects were used for this cohort, it may be suggested that these intakes are representative of a daily dietary intake for AMD patients who seek the services of the macular society, as a three-day dietary recall provides a more accurate illustration of habitual diet.

Lutein and Z:

This sample of subjects with AMD consumed an average of 1.7 mg of L and Z per day. This is considerably lower than the 10 mg daily amount considered to increase macular pigment optical density (MPOD) and delay the progression of AMD [219], [99], [103], [220], [71]. These findings correspond with a previous study carried out by our research group using a 24 hour recall to signify the diet of AMD patients who are members of the UK Macular Society. In this study reported average intakes of dietary L and Z were 1.6 mg per day [211]. L&Z consumption was compared with age, gender, AMD type (dry vs. wet), the number of eyes affected, perceived vision and visual registration status, but no trends were apparent. Despite 81% of subjects not being on a visual impairment register as sight impaired or severely sight impaired (blind), 74% of subjects claimed that their vision was average or below on the day of the survey and 65% of patients had AMD in both eyes. This may indicate that patients have not registered their visual impairment. The RNIB's survey into certification and registration in 2011 [221], shows a steady decline in the number of registrations per year (a fall of 30% for new blind registrations, and a 28% decrease in new sight impaired registrations from 2003 to 2011), despite the fact that the prevalence of visual impairment is increasing. They attribute this decrease in registration not to a lack of interest in registration by the patient, but rather to the length of time it takes to complete the Certificate of Visual Impairment (CVI) by the professionals involved [222].

Calories and Carbohydrates:

Results demonstrate that both males and females failed to meet the government DRVs for calorie intakes. The mean consumption of daily calorie intake was 1594 kcal, much lower than the minimum DRV of 1840 kcal recommended for those over the age of 75 years (mean age of cohort). Similarly, mean intakes of calories specifically for those aged 75 or older were 1660 kcal for females, and 1523 kcal for males, both significantly lower than the DRV ($p<0.001$). In the previous 24 hour recall study average daily calorie intakes for AMD patients were 1511 kcal [211]. An average intake of 202.7 g per day of carbohydrate (80.3 g of sugars) was reported which was much lower than the government DRV of 230 g per day. Carbohydrate intakes were found to be significantly lower (independent t test =0.036, $p <0.05$) in those with AMD in both eyes compared with those with AMD in one eye. This decline in food intake presumably follows in part from a decrease in physical activity and the decline in muscle mass with age. This results in a lower requirement for energy (substantial reductions in energy intake may, in turn, result in lower physical activity,

a declining cycle) and thus a diminished appetite. This trend may be more apparent in those with more advanced stages of AMD as visually impaired patients are less physically active and more likely to have mobility limitations than individuals with normal visual capabilities [223], [224]. Other physical and psychosocial barriers which may exacerbate diminished appetite within this vulnerable population may also play a part, such as living alone [225], [226], poor cooking skills and the inability to cook and prepare foods [211]. Potential problems arise because, as total food intake declines, for most nutrients there is a simultaneous decline in nutrient intake. This was evident in the current study which indicated that subjects were also not attaining the DRV of other nutrients important for their condition such as L/Z, vitamin E, vitamin D and zinc, and nutrients important for general health such as fibre, potassium and vitamin A in males.

It is important to note that other surveys of the UK population as a whole [227], [228] show that the mean Body Mass Index (BMI) and number of people classified as overweight or obese is increasing. The latter data indicate that average habitual energy intake may exceed energy needs. Reported intakes of food energy being lower than intakes actually consumed may be the reason behind this apparent paradox [229], [230]. In particular, under-reporting of foods during dietary assessment is significant in people who are overweight and obese [231], [232], [233]. Whether this may be the case in the present AMD cohort is unknown, data on body weight or body mass within this population may be required to support an accurate representation of calorific intakes.

Other Nutrients with Clinical Significance to AMD

Selenium and Vitamin E:

Selenium and vitamin E were also below the DRV within this cohort despite foods rich in vitamin E being recommended by the MS in their patient resources. These nutrients have been implicated in the protection of biological membranes against lipid peroxidation, a process that may be induced either by production of metabolic by products such as reactive oxygen species (ROS) or by exposure to atmospheric oxidants [234], [235]. The eye is particularly susceptible to lipid peroxidation due to the high concentration of fatty acids [61]. Vitamin E, in the form of alpha-tocopherol [58], [59] is predominant in the retina where it is the major lipid-soluble antioxidant of all membranes and the most effective scavenger of ROS [57] [60]. Evidence supporting an oxidative pathogenesis of AMD has increased interest in the potential

preventative role of nutritional supplementation with dietary antioxidants such as vitamin E.

Vitamin D:

Vitamin D levels for both males and females in the cohort were significantly below the DRV (independent t test $p < 0.001$). Vitamin D deficiency is common in the elderly due to limited sun exposure because of changes in lifestyle factors such as clothing, outdoor activity and dietary habits. Vitamin D deficiency in this population may also be attributed to a reduced amount of vitamin D precursor in the skin and diminished renal function [236]. Exposure to sunlight, which enhances the production of vitamin D_3 in the skin, is important as a limited number of foods contain vitamin D [237], [238]. Vitamin D deficiency is also common amongst ethnic minority groups in the UK such as Asians whose origins are from India, Pakistan, or Bangladesh as certain cultural habits such as covering the skin may restrict ultraviolet irradiation [239, 240]. Deficiency of vitamin D results in abnormalities in calcium, phosphorus, and bone metabolism, as absorption of dietary calcium and phosphorus is decreased. It is characterised by mild secondary hyperparathyroidism and an enhanced risk of osteoporotic fracture [241], [242], [243]. In the eye, vitamin D has anti-inflammatory properties which may suppress the cascade of destructive inflammation that occurs at the level of the retinal pigment epithelium-choroid interface in early stages of AMD. There are several epidemiological studies suggesting an association between vitamin D deficiency and AMD [244], [245], [246], although currently the MS do not include details of Vitamin D in their resources. This is because few have tested the effect of vitamin D supplementation on the prevention and treatment of AMD. Research does suggest that the recommended intakes for vitamin D are inadequate, and, in the absence of exposure to sunlight, a minimum of 1000 IU vitamin D is required to maintain a healthy serum concentration [247], [248].

Vitamin C:

The mean intake of vitamin C was 121 mg/per day, which was significantly higher (one sample t test $p < 0.001$) than the DRV of 40 mg per day for men and women over the age of 18 years. Vitamin C is water-soluble and is involved with several biological processes. It has been shown to have antioxidant properties which enable it to react directly with hydroxyl radicals [64], superoxide [65] and singlet oxygen molecules [66]. High dose supplementation with an antioxidant and zinc formulation, including vitamin C has been associated with a 25% reduced risk of progression of AMD in those

participants already suffering with the condition (AREDS 1) [49]. Low levels of vitamin C in the body have also been associated with an increased risk of AMD [72]. Some studies, however have found no evidence for a beneficial role of vitamin C supplementation alone within AMD [73], [74], [75]. It is therefore not known whether it can slow the progression of AMD when not in combination with other antioxidants. Despite this, the MS encourage inclusion of foods rich in Vitamin C in the diet which may explain the reason for an increased consumption within this cohort of subjects.

Zinc:

Zinc has been investigated regarding its potential preventative role in AMD. The AREDS group found a suggestive reduction in the risk of progression of AMD in participants supplementing with 25 mg zinc daily [71], which is a key research finding the MS discuss in their nutritional information for patients. A small number of cohort studies assessing dietary intakes of zinc in elderly subjects have found an inverse relationship between high zinc intake and the incidence of early AMD. [175], [150]. However other large cross–sectional studies involving zinc and the progression of AMD have yielded conflicting results [7], [179]. Female subjects within this cohort consumed dietary zinc at levels above the government DRV of 7.0 mg per day [7.9 (95% CI 7.4-8.4)] whereas male subjects were under consuming the DRV of 9.5 mg per day [9.0 (95% CI 8.4-9.6)]. This may be explained by an increased awareness of research into zinc supplementation and the condition within women subjects.

Limitations

The current study contains several methodological limitations. Firstly, dietary intake accuracy based on 24-hour recalls is influenced by memory errors and could result in over reporting or underreporting of food intake, particularly among elderly populations [214, 249]. To improve accuracy of data and give a more reliable estimation of habitual intakes, three non-consecutive days of 24 hour recalls were conducted. There was a lack of information regarding subjects BMI and activity levels, which would have given further insight in to the accuracy of calorific intake. Participants were not required to weigh their food during recording; therefore portion sizes were estimated from household measures and published food reports which may have resulted in some misreporting. Subjects were also told in advance of what day a recall was due to be conducted which may have led to bias as previous reports have found that individuals may over or under report “good/bad” food items [214, 250]. Finally, the sample used in this study may not be reflective of all AMD patients as only participants who were already part of the society and those who called the helpline

were invited to take part. Selection bias should therefore be considered as patients who have sought the help of the MS could be considered an 'informed' population as they have information available to them in the form of monthly magazines, written material, a helpline and the Society's website. It is also important to highlight that a large proportion of the cohort were female and over the age of 75 years old and there was a lack of information regarding subject's ethnicity. It would be beneficial to gain further dietary information from those with AMD from younger populations, of both sexes and varied ethnicities, who have not sought support from non-professional organisations.

2.5 Summary

This study supports the evidence that patients with AMD who seek the services of the MS are under consuming important nutrients for their condition. The reasons for this are likely to be multi-factorial. There may be several barriers to dietary change within this cohort of visually impaired patients in addition to the general individual variances of food choice and preferences. In a previous study, our research group looked further in to the barriers to why AMD patients who seek the services of the MS are under consuming important nutrients by determining their awareness of the relationship between nutrition and AMD [210]. It was documented that over half (56%) of AMD subjects could acquire food themselves, and used a supermarket as a primary source for acquiring food sources. However, the remaining 44% of patients were unable to shop for themselves. When considering patients' food choices, the largest influencer appeared to be preference (44%), with the next largest (33%) being foods that the subjects believe would affect their health. The ability to prepare the foods was a factor for 6% of subjects, and 3% said that the ability to acquire the food was the predominant influencer. Further research from a survey into independent living for the RNIB [251] indicated that 'preparing a meal' was one of the most commonly identified challenging task by visually impaired people (33%). When asked how they acquire their food, 56% of the participants said that they themselves were able to go food shopping; however, 28% relied on a family member, 3% had a friend and 2% had a caregiver who went food shopping for them and 12% had another method of acquiring food (this could be the internet, meals on wheels etc). Although subjects who seek the services of the MS may be considered an informed population as they receive dietary advice from the MS, altering long term dietary habits may be challenging in these older populated groups when there are underlying barriers to

change. Strategies to ensure that nutritional information and interventions consider possible multi-factorial barriers within this vulnerable group may be needed.

This study investigated the dietary habits in a group of AMD patients to establish whether subjects were regularly consuming nutrients regarded as important for their condition such as L and Z. It was concluded that further research is needed to discover the potential barriers to dietary change within visually impaired populations. The following chapter will describe a study which aims to investigate the optimum pre- and post-harvest conditions for maximisation of L in kale. Findings from this study will enrich the current dietary advice given to AMD patients, and serve as a foundation to the development of novel intervention which aims to improve the overall diet of those suffering with AMD.

Chapter 3: Determination of the optimum pre and post-harvest conditions for the maximisation of lutein concentrations in kale

3.1 Background and rationale

The two major carotenoids in the human macula and retina are L and Z [252], which are often referred to as xanthophylls which form the MP. In the eye carotenoids such as L function as antioxidants and blue light filters to protect underlying tissues from phototoxic damage [50]. Fruits and vegetables are the most important source of carotenoids in the human diet and knowledge about this is important for preventive medicine. Dark green leafy vegetables such as kale and spinach are recognised as containing the highest amounts of dietary L [253]. Lutein and Z dietary intake is implicated in the maintenance of retinal health and possible prevention of the onset or progression AMD [254]. Currently AMD patients are advised to include more dark green vegetables such as kale in to the diet to increase their intake of L and Z. However, little is known about the pre-and post-harvest effects on the content and bioavailability of L and Z concentrations within fruits and vegetables. Combined L/Z levels in the leaves of kale are reported to range from 147 to 395 μg−1g of fresh tissue [94] as carotenoid accumulations in plants are often influenced by physiological and biochemical attributes such as processing and storage as well as environmental growth factors such as light, temperature and fertility [255] [196], [256]. Although some studies have looked in to the pre- and post-harvest effects on the carotenoid concentrations in spinach [257-260], there are very few studies to date which have investigated the post- harvest processing (cooking) and storage effects on kale L concentrations. Furthermore, no studies to date have investigated the L levels in kale grown and sourced within the UK. Therefore this study was designed to answer these questions.

Kale is a vegetable of the plant species Brassica Oleracea. It has green or purple leaves, in which the central leaves do not form a head. It is considered to be closer to wild cabbage than most domesticated forms of vegetables. Kale is produced in the UK nearly all year round as it is a hardy, resilient plant which tolerates cold whether much better than other brassicas. According to industry source (www.discoverkale.com; January 2016) most of the kale in the UK which is supplied to supermarkets across the country is grown and produced on open fields in Lincolnshire farms between June and March, with the late autumn and early winter months when it is at its most plentiful. The first kale of the year is planted at the

beginning of April, and in order to provide a consistent supply of fresh young produce, sequential plantings are made through until August. Further information about the pre and post-harvest procedures of UK kale manufacturing was gathered from a day visit to one of the Lincolnshire farms (Emmet UK Ltd). It was noted that kale plants take two to three months to grow depending on the outside temperature and the early seeds are cultivated in greenhouses for the first few weeks to speed up their growing process. Once harvested, the kale is sliced and washed in water and citrox (fruit acid) for 90 seconds and dried and packaged ready for transportation to supermarkets around the UK. The packaging is done immediately after washing, in vacuum or in a gas mixture of 20% CO_2 + 80% N_2. The process from harvest to packaging takes around 24 hours. Kale is then in dispatch for up to 12 hours under refrigeration in dark at 4-5 °C. Once it reaches the supermarket it has a shelf life of 5 days at 8°C. This 'fresh-cut' process (a term which refers to raw vegetables and fruits that have been cut, shredded, peeled, abraded, or otherwise prepared and packaged to produce convenient ready-to-eat or ready-to-cook portions) [261] is a current trend for marketing fruits and vegetables, stimulated by increasing consumer demand for high-quality, nutritive, fresh-like and convenient-to-use products.

The aim of the present study was to determine the optimum conditions under which kale, the vegetable that contains the highest concentration of L and Z, can be acquired, stored and domestically processed for maximum retinal benefit. Due to cost implications, only L concentrations within kale were investigated. Zeaxanthin is required to support retinal function at much lower doses than lutein and therefore was not investigated within the study. Lutein levels were identified by high-performance liquid chromatography (HPLC) in the leaves of freshly harvested kale from a UK Lincolnshire farm along with kale acquired from a UK supermarket where the same farm supplies to, at different times of the year. Both varieties of kale were used to compare the effects of post-harvest minimal processing and sourced throughout different times of the year to determine the pre-harvest climatic effects on caroteneogensis. Kale was further investigated in relation to its L stability upon domestic processing procedures such as boiling, blanching, steaming, frying, microwaving and liquidising. In addition, L concentrations in freshly harvested and supermarket purchased kale were analysed upon various fridge and freezer storage periods. Results from the study will provide patients and clinicians with a clearer understanding of the effects of pre-and post-harvest conditions on the carotenoid content of vegetables which will enable precise suggestions for increasing retinal

levels of these nutrients. Dr Val Franklin, a collaborator from the University with expertise in HPLC was appointed to assist with this project.

3.2 Methods

Method of preparation techniques for kale samples

The first stage of the analysis included preparation and cooking techniques which enabled the concentration of L to be extracted and then identified by HPLC. High-performance liquid chromatography is a form of liquid chromatography to separate compounds that are dissolved in a solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector [262]. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behaviour between the mobile liquid phase and the stationary phase which is known as their retention time. The HPLC system then produces a chromatogram image on the computer which displays a series of peaks over different retention times for each compound. This is known as a calibration curve and is a general method for determining the concentration of a substance in an unknown sample by comparing it to a set of standard samples of known concentration. For example, L concentrations within samples were quantified by determining the peak area in the HPLC chromatogram calibrated against a known amount of L standard. Standards of L were processed at different concentrations first to produce the calibration curve which enabled the quantity within the sample to be determined.

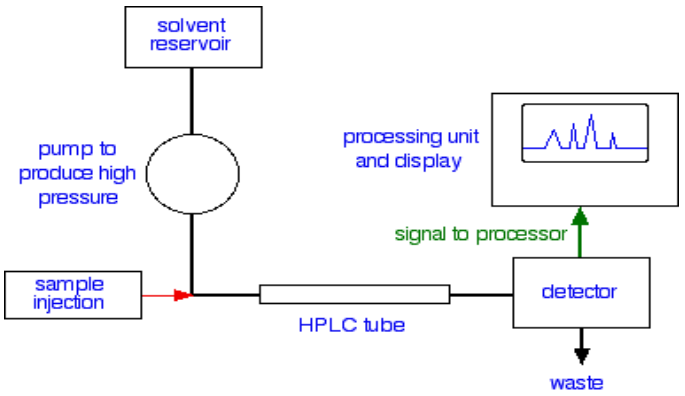

Figure 2.4: A schematic diagram representing the HPLC process.

A portion of freshly harvested kale leaves were delivered from a main UK industrial supplier (harvested and delivered within 24 hours, before its shelf life) over the months of March (Spring), August (Summer), October (Autumn) and January (Winter). Kale leaves were also purchased from a local UK supermarket, within its shelf life constituency on the same day of each specific month. Within 24 hours of delivery and purchase, the samples of freshly harvested and shop purchased kale were prepared for analysis in the common manner, which included hard stems and blemishes being removed.

Figure 2.5: Freshly harvested kale delivered from the farm within 24 hours ready for preparation and L analysis by HPLC.

One sample of each kale variety was retained raw and the rest were processed in various methods to determine the containing L stability via HPLC. These methods included steaming, boiling, blanching, microwaving, frying and liquidising. Lutein concentrations in kale samples were also quantified after a range of storage treatments, which included conventional fridge and freezer storage over various periods of time. Each treatment was performed in four replicates to increase reliability.

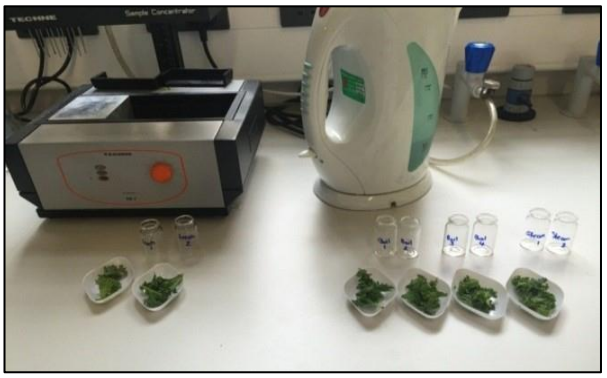

Figure 2.6: Kale samples equally weighed ready for processing in their labelled containers.

Method of processing techniques for kale samples

The first analyses were carried out in spring where L concentrations of raw kale of each variety along with three common domestic cooking techniques (steaming, boiling and frying) were determined. This pilot investigation was needed to identify any potential issues with the methodology. During these initial stages frying was performed using a conventional cooking method with a small amount of olive oil (2ml per 1g sample). Due to lower than expected readings and difficulty measuring L within the lipid structure, kale was fried without the use of oil in future analyses (summer, autumn, winter). Technical issues with the steaming equipment were also noted during the pilot investigation, thus future analyses using this process were not carried out.

Additional methods for cooking and processing kale were included in the summer, autumn and winter months. These included microwaving kale using three techniques (micro 1: 1 minute with a dessert spoon of water, micro 2: 2 minutes with a dessert spoon of water, micro 3: 2 minutes with a tablespoon of water) and liquidising kale with a small amount of water (2ml per 1g sample). Blanching kale was investigated as an alternative method to boiling kale in the winter to establish whether or not a shorter duration of exposure to boiling water would be better at retaining L concentrations. Blanching may be described as the process whereby vegetables are immersed in boiling water and then removed after a brief, timed interval to halt the cooking process. The ratios of water to kale were

Various fridge and freezer storage periods were also included in the post spring analyses to determine the storage effects on L concentrations in kale. After all preparation and cooking techniques were completed, samples were ready for extraction and analysis by HPLC.

Fresh (spring, summer, autumn, winter)	Boiling (spring, summer)	Steaming (spring)	Frying with oil (spring)
Within 24 hours of collection, four replicates of each kale variety were weighed in to 1g samples and kept fresh without processing	Within 24 hours of collection, four replicates of each kale variety were weighed in to 1g samples and placed in a container of 10ml of boiling water for 3 and 5 minutes. Each container was covered to prevent evaporation of water	Within 24 hours of collection, four replicates of each kale variety were weighed in to 1g samples and steamed using a conventional steamer for 4 minutes over 4ml of water	Within 24 hours of collection, four replicates of each kale variety were weighed in to 1g samples and fried for 10 minutes in 2ml of olive oil. They were then dabbed with blotting paper to absorb excess oil

Microwaving (summer, autumn, winter)	Frying no oil (summer, autumn, winter)	Liquidising (summer, autumn, winter)	Fridge storage (summer, autumn, winter)	Freezer storage (summer, autumn, winter)
Within 24 hours of collection, four replicates of each kale variety were weighed in to 1g samples and placed in a microwavable bowl for 1 minute and 2 minutes with a dessert spoon of water (micro 1 and 2) and for 2 minutes with a tablespoon of water (micro 3). Each container was covered to prevent water loss due to evaporation	Within 24 hours of collection, four replicates of each kale variety were weighed in to 1g samples and fried in a small pan on a hot plate without oil for 5 and 10 minutes	Within 24 hours of collection, four replicates of each kale variety were weighed in to 1g samples and liquidised for 1 minute with a 2ml of water in a conventional smoothie maker	Within 24 hours of collection, four replicates of each kale variety were retained raw and weighed in to 1g samples ready for fridge storage. Samples were placed in a bag and sealed then stored in a fridge at 4°C for four days and seven days.	Within 24 hours of collection, four replicates of each kale variety were retained raw and weighed in to 1g samples ready for freezer storage. Samples were placed in a bag and sealed then stored in a freezer at -18°C for seven days and twenty-eight days

Table 2.9: Spring, summer, autumn and winter L analyses: processing and storage techniques.

Selection and modification of the extraction and HPLC technique

A review of the literature was carried out on the Web of Science, PubMed and Science Direct databases to identify relevant studies that had investigated extraction and HPLC analysis of L from food sources. Search terms used were 'lutein', 'HPLC' and

'analysis'. We identified articles that reported on L quantification of vegetables through extraction and HPLC analysis techniques published in peer-reviewed journals. The chosen method for the extraction and HLPC technique was taken from a study by Chung, H Rasmussen, H and Johnson, E (2004), as it was well cited in the literature and fit best with the equipment we had available [96]. The only modification that was made to the method was using micro bore columns for the injection process, thus the sample weight and solvents were reduced proportionally to accommodate for the present HPLC system.

Extraction process:

The first stage of extraction once the kale samples had been processed in the various methods included incubating the samples in methanol overnight for 16 hours. Carotenoids are lipid soluble molecules and therefore usually extracted from a plant source with water-miscible organic solvents. To obtain a safe extract, elimination of the residual solvents can be avoided by using food grade organic solvents such as methanol [263]. Firstly samples were homogenised using a pestle and mortar until ground down. 5ml of methanol was then added to each 1g of sample in a 50ml glass vial. Sample containers were labelled and covered with tin foil and para fill and incubated overnight for 16 hours in a fridge at 4°C.

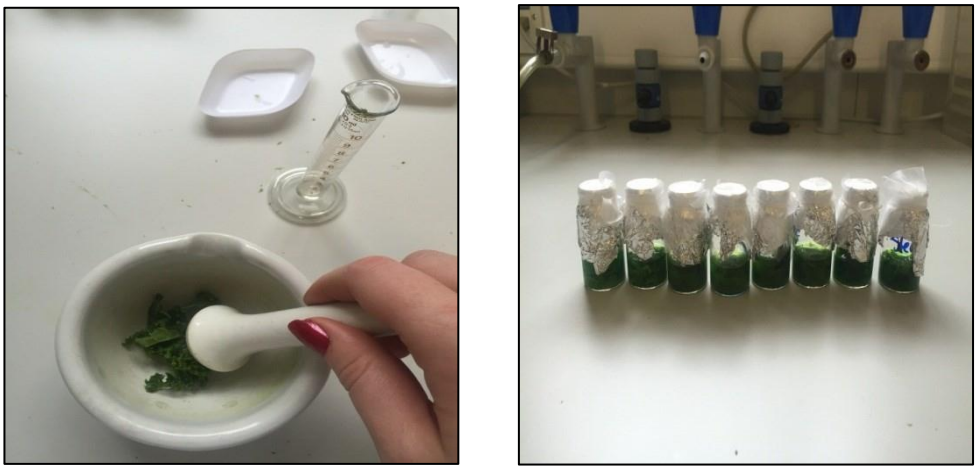

Figure 2.7: Kale samples being grinded up ready for the additional of methanol (solvent 1); kale samples after the first stage of extraction ready for incubation.

The second stage of extraction included the addition of the second solvent tetrahydrofuran (THF). The incubated samples were centrifuged at 800xg for 10min and then the methanol layer was removed into separate glass containers using a

Pasteur Pipet. 5ml of THF was added to each vial, vortexed for 30 seconds and then centrifuged at 800xg for 2 minutes. The THF layer was then added to each methanol container. This extraction process with THF was repeated four more times adding a THF layer to the methanol container each time. A sufficient amount of THF was added to make the final volume up to 25ml. 10ml of each sample of mixed solvent was dried under nitrogen to concentrate the sample and preserve the active ingredients. 100ul of the mixed solvent was re dissolved in to a dried down sample. A pipette was used to extract each re dissolved sample in to an amber vial. Samples were then ready to inject on to the HPLC column.

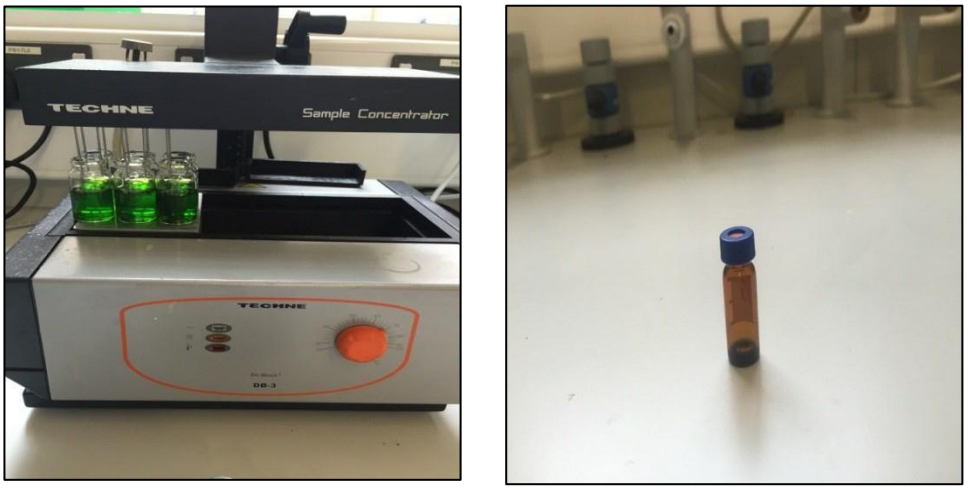

Figure 2.8: Kale samples after the second stage of extraction; one sample upon completion of the extraction process ready to be injected on the HPLC column.

HPLC analysis:

To adequately separate the L a HPLC system comprised of a Waters 600S controller (Millipore), Waters 616 pump, Waters 717 autosampler, Wavelength 44nm photodiode array detector, and C30 carotenoid column (3 µm, 150 × 4.6 mm, YMC) was used. The HPLC mobile phase was methanol: MTBE: water (95:3:2, v/v, with 1.5% ammonium acetate in water, solvent A) and methanol: MTBE: water (8:90:2, v/v, with 1.0% ammonium acetate in water, solvent B). The gradient procedure, at a flow rate 0.4 mL/min (10 8C), was as follows: (1) start at 100% solvent A, (2) a 21-min linear gradient to 45% solvent A and 55% solvent B, (3) 1-min hold at 45% solvent A and 55% solvent B, (4) an 11-min linear gradient to 5% solvent A and 95% solvent B, (5) a 4-min hold at 5% solvent A and 95% solvent B, (6) a 2-min linear gradient back to 100% solvent A, and (7) a 28-min hold at 100% solvent A.. Lutein was quantified by determining the peak area in the HPLC chromatogram calibrated against a known amount of standard. Peak identification in samples was based on

comparisons with retention time and absorption spectra of the carotenoid standard of L.

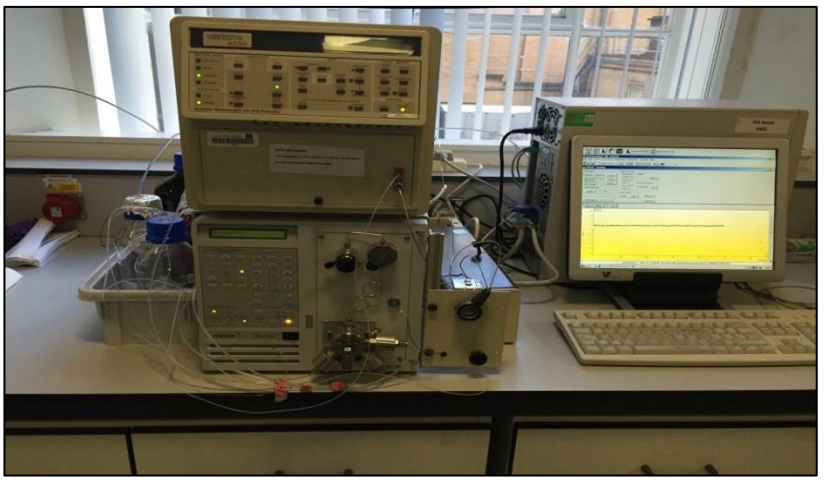

Figure 2.9: HPLC system used in the study.

3.3 Results (effects of minimal processing and season)

The first section of results describes the variation in L content of raw kale sourced from both the farm and supermarket. Freshly harvested kale was sourced from a farm in Lincolnshire and delivered for analysis within 24 hours of harvest. The kale was classified as being intact whole produce that had not been minimally processed, a technique used for retail purposes which includes cutting, washing and modified atmosphere packaging. Minimally processed kale was purchased from a retailer which the same farm supplies to within its five-day shelf life constituency. Prior to delivery to its retail outlets the kale was washed, sliced and packaged in 200g units and sold within five days. Kale was delivered to the supermarket within 24 hours of harvest. Four raw sample lots for each season (spring, summer, autumn and winter) were collected at different times during the season and analysed individually. All samples were analysed immediately after collection.

Shop (mg/100g)				
Spring	Summer	Autumn	Winter	
13.41	10.64	10.54	11.74	
13.39	10.65	10.5	11.73	
13.41	10.63	10.54	11.73	
13.40	10.63	10.54	11.73	
13.40	**10.64**	**10.54**	**11.73**	**Mean**
0.004	**0.010**	**0.004**	**0.003**	**SD**

Farm (mg/100g)				
Spring	Summer	Autumn	Winter	
20.30	21.62	21.52	21.98	
20.31	21.62	21.53	21.99	
20.31	21.62	21.53	21.98	
20.30	21.62	21.53	21.98	
20.31	**21.62**	**21.53**	**21.98**	**Mean**
0.006	**0.003**	**0.005**	**0.003**	**SD**

Table 3.1: Lutein levels in mg/100g for raw kale sourced from both the supermarket and farm during various times of the year.

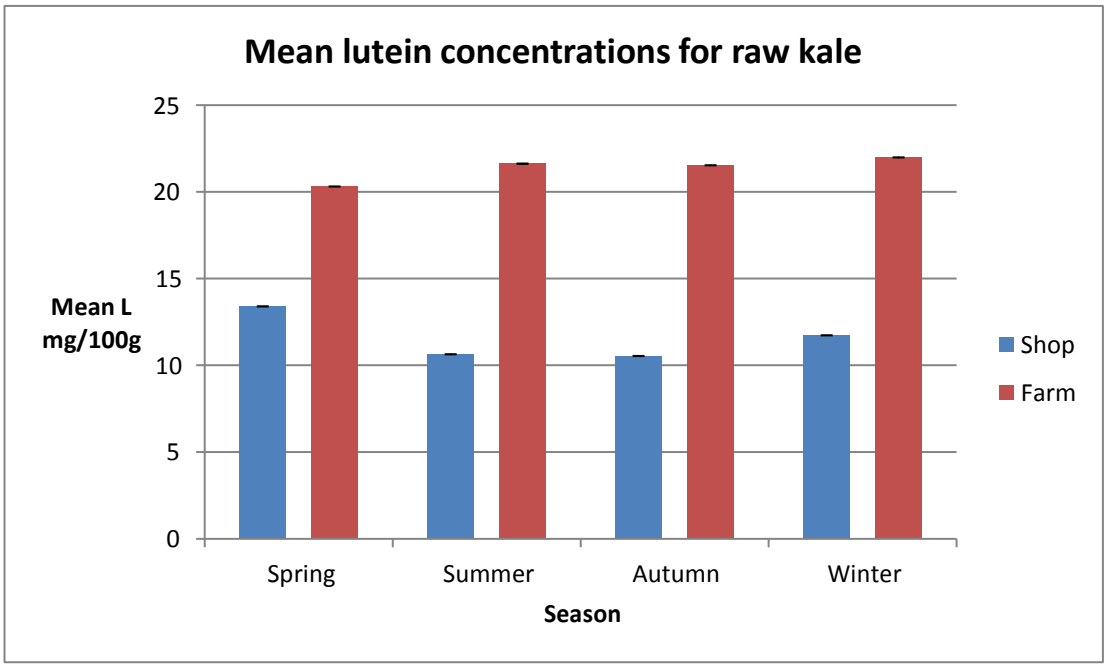

Figure 3.1: Difference in lutein levels between raw farm and shop kale upon various times of the year using the mean values.

Table 3.1 and figure 3.1 display data on L levels in raw kale of each variety (farm and shop) throughout different periods of the year. Four replicates of each HPLC determination were performed for both kale varieties at these seasonal intervals and the raw data is expressed in table 3.1. Figure 3.1 displays the L concentrations of raw farm and shop kale expressed as mean values.

Statistical Analyses

Data for L levels in raw freshly harvested and minimally processed kale at different times of the year were analysed in statistical software IBM SPSS version 20 (IBM UK Ltd, Portsmouth, Hampshire) to draw comparisons between results using parametric and non- parametric tests. Normality of data was evaluated using a Kolmogorov-Smirnov and Shapiro-Wilk Test. Mean values were considered significantly different at $p < 0.05$. To determine the difference between L concentrations of raw minimally processed and freshly harvested kale, a Mann- Whitney U test was used. It was found that kale purchased from the supermarket within its shelf life constituency had statistically significantly lower L concentrations (11.58 ± 1.12 mg/100g) compared to kale sourced direct from the farm within 24 hours of harvest (21.4 ± 0.65 mg/100g), $Z = -4.826$, $p < 0.001$.

The differences between the L concentrations of raw shop and farm kale at different times of the year (spring, summer, autumn, winter) were detected by a two-way ANOVA. There was a statistically significant main effect for kale source [$F(1,429) = 27792837.4$, $p<0.001$] and kale season [$F(1,429) = 58614.2$, $p<0.001$] and the effect size was large for both factors (eta squared = 1.00). The interaction effect between kale source and kale season was significant (season*source: sig.=<0.001), therefore follow up statistical tests were used to explore this relationship further. The file was split by kale source to investigate the effects of season separately by a one-way ANOVA. A statistically significant difference was found between all four seasons for shop kale ($p<0.0001$) and for farm kale ($p< 0.0001$). Despite this, the actual difference in the mean values was very small, particularly for farm kale, as observed in the plot graph. For example, the mean L values for farm kale were 20.31, 21.62, 21.53, and 21.98 mg/100g in spring, summer, autumn and winter respectively.

3.4 Results (effects of cooking and storage)

The second section of results describes the effects of various domestic processing methods (boiling, steaming, stir frying, microwaving, blanching, liquidising) and fridge and freezer storage on the L levels in freshly harvested and minimally processed kale. Freshly harvested kale was sourced from a farm in Lincolnshire and delivered for analysis within 24 hours of harvest. The kale was classified as being intact whole produce that had not been minimally processed, a technique used for retail purposes which includes cutting, washing and modified atmosphere packaging. Minimally processed kale was purchased from a retailer which the same farm supplies to within

its five-day shelf life constituency. Prior to delivery to its retail outlets the kale was washed, sliced and packaged in 200g units and sold within five days. Kale was delivered to the supermarket within 24 hours of harvest. Four replicates of each treatment were performed and results were presented as mean values. All samples were analysed immediately after collection.

Sample/Treatment	**Spring (n=4)**	**Summer (n=4)**	**Autumn (n=4)**	**Winter (n=4)**	**Mean**	**SD**
	L (mg/100g)	L (mg/100g)	L (mg/100g)	L (mg/100g)	L (mg/100g)	
Fresh	13.40	10.64	10.54	11.73	11.58	1.15
Steam	9.88				9.88	
Boil 3 min	6.46	6.38	6.32		6.38	0.06
Boil 5 min	3.79	3.86	3.79		3.81	0.03
Blanch				9.97	9.97	0.00
Fried (oil)	2.02				2.02	
Fried (no oil) 5 m		4.76	4.70	4.83	4.76	0.05
Fried (no oil) 10 m		3.76	3.98	4.06	3.93	0.13
Micro 1		7.34	7.24	7.38	7.32	0.06
Micro 2		5.35	5.20	5.43	5.33	0.09
Micro 3		5.20	5.13	5.31	5.21	0.08
Liquidise		9.37	9.15	9.42	9.31	0.12
Fridge 4 days		6.20	6.16	6.20	6.19	0.02
Fridge 7 days		4.44	4.36	4.42	4.40	0.04
Freezer 7 days		7.20	6.98	7.34	7.17	0.15
Freezer 28 days		4.38	3.99	4.23	4.20	0.16

Table 3.2: Mean L concentrations in minimally processed kale after various processing and storage procedures throughout different periods of the year.

Sample/Treatment	Spring (n=4)	Summer (n=4)	Autumn (n=4)	Winter (n=4)	Mean	SD
	L (mg/100g)	L (mg/100g)	L (mg/100g)	L (mg/100g)	L (mg/100g)	
Fresh	20.31	21.62	21.53	21.98	21.36	0.73
Steam	11.50				11.50	
Boil 3 min	6.64	6.79	6.74		6.72	0.08
Boil 5 min	3.94	3.89	3.83		3.89	0.05
Blanch				11.69	11.69	
Fried (oil) 5 m	2.57				2.57	
Fried (no oil) 5 m		4.89	4.83	4.99	4.90	0.08
Fried (no oil) 10 m		3.89	4.07	4.29	4.08	0.20
Micro 1		9.63	9.52	9.73	9.63	0.11
Micro 2		6.83	6.70	6.84	6.79	0.08
Micro 3		6.33	6.28	6.34	6.32	0.03
Liquidise		16.81	16.60	16.73	16.71	0.10
Fridge 4 days		8.80	8.76	8.85	8.80	0.04
Fridge 7 days		5.04	4.98	5.33	5.11	0.19
Freezer 7 days		9.63	9.47	9.80	9.63	0.17
Freezer 28 days		6.25	5.99	6.29	6.18	0.16

Table 3.3: Mean L concentrations in freshly harvested kale after various processing and storage procedures throughout various periods of the year.

Tables 3.2 and 3.3 display the mean L concentrations in minimally processed and freshly harvested kale after each processing and storage treatment. Four replicates of each analysis were performed and the data were presented as mean readings. Further mean readings were reported of the L concentrations of kale samples across the year. A pilot analysis was carried out in spring where raw kale of each variety along was investigated in relation to three common domestic cooking techniques, which included steaming, boiling and frying. During this pilot investigation, frying which was performed using a conventional cooking method with a small amount of olive oil (2ml per 1g sample) was found to be the most detrimental of the methods. However, the values obtained should be taken with caution as L is a fat-soluble molecule and it is likely that the low amounts reported were related to the active being retained in the oil. Due to lower than expected readings and difficulty measuring L within the lipid structure, kale was fried without the use of oil in future analyses. Boiling, particularly for a longer duration of five minutes, also had negative effects on L levels in both varieties of kale therefore blanching was used as an alternative method of cooking in the winter analyses. Additional methods for cooking and processing kale in the summer, autumn and winter periods included microwaving kale using three techniques (micro 1: 1 minute with a dessert spoon of water, micro 2: 2

minutes with a dessert spoon of water, micro 3: 2 minutes with a tablespoon of water) and liquidising kale with a small amount of water (2ml per 1g sample) from raw. Various fridge and freezer storage periods were also included in the post spring analyses to determine the storage effects on L concentrations in kale.

Shop Kale	Mean L mg/100g	% reduction of L from raw
Steam	9.88	14.80%
Boil 3 min	6.38	44.91%
Boil 5 min	3.81	67.10%
Blanch	9.83	15.11%
Fried (oil)	2.02	82.56%
Fried (no oil) 5 min	4.76	58.89%
Fried (no oil) 10 min	3.93	65.63%
Micro 1	7.32	36.79%
Micro 2	5.33	53.97%
Micro 3	5.21	55.01%
Liquidise	9.31	19.60%
Fridge 4 days	6.19	46.55%
Fridge 7 days	4.40	62.00%
Freezer 7 days	7.17	38.80%
Freezer 28 days	4.20	63.73%

Table 3.4: Percentage in reduction of L concentrations from raw supermarket sourced kale after each processing and storage treatment.

Farm Kale	Mean L mg/100g	% reduction of L from raw
Steam	11.50	46.16%
Boil 3 min	6.72	68.54%
Boil 5 min	3.89	81.79%
Blanch	10.85	49.20%
Fried (oil)	2.57	87.97%
Fried (no oil) 5 min	4.90	77.06%
Fried (no oil) 10 min	4.08	80.09%
Micro 1	9.63	54.92%
Micro 2	6.79	68.21%
Micro 3	6.32	70.41%
Liquidise	16.71	21.77%
Fridge 4 days	8.80	58.80%
Fridge 7 days	5.11	76.08%
Freezer 7 days	9.63	54.92%
Freezer 28 days	6.18	71.07%

Table 3.5: Percentage in reduction of L concentrations from raw farm sourced kale after each processing and storage treatment.

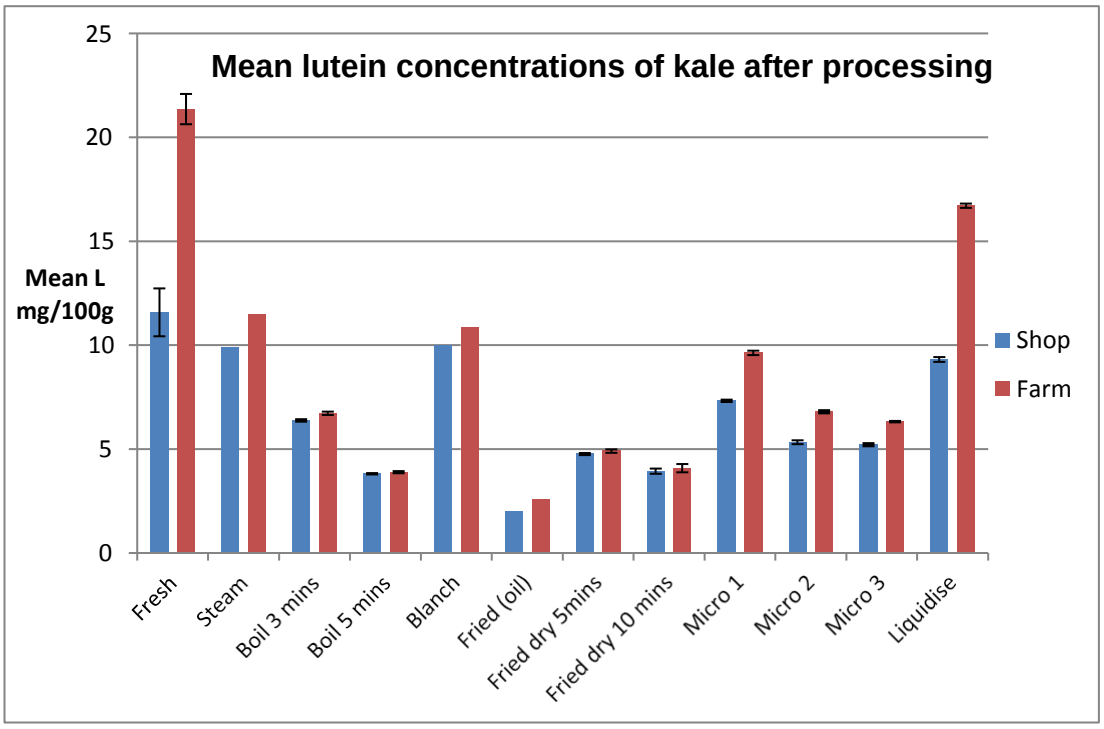

Figure 3.2: Mean L concentrations of supermarket sourced and farm sourced kale upon various processing procedures.

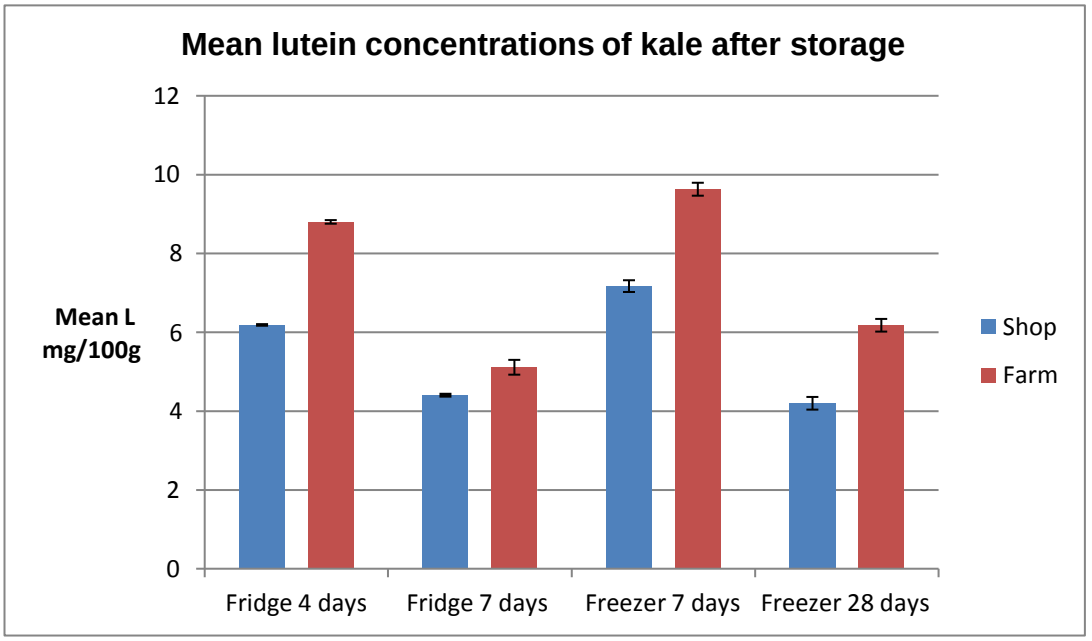

Figure 3.3: Mean L concentrations of supermarket sourced and farm and sourced kale upon various storage procedures.

Statistical Analyses

Data for L levels after various cooking and storage procedures were analysed in statistical software IBM SPSS version 20 (IBM UK Ltd, Portsmouth, Hampshire) to draw comparisons between results using parametric and non- parametric tests. Normality of data was evaluated using a Kolmogorov-Smirnov and Shapiro-Wilk Test. Mean values were considered significantly different at $p < 0.05$. Statistical comparisons were made between lutein levels in raw and cooked samples, and between individual cooking and storage techniques. These differences were detected by independent t-tests and Mann-Whitney U tests depending on the normality of data. Differences were considered to be significant at $p < 0.05$.

3.5 Discussion (effects of minimal processing and season)

The study described here explored the L levels in raw freshly harvested kale sourced from the farm along with minimally processed supermarket purchased kale, at various times of the year. As a possible preventer of AMD, efforts to increase MP levels through simple dietary methods such as increasing dark green leafy vegetables in the diet are important. Gathering further information about the pre-and post-harvest effects on the L levels in kale is needed, thus setting a precedent for this study.

The L concentrations in mg/100g of kale as affected by source and season are shown on table 2.3. Lutein levels differed significantly ($p<0.001$) between kale which had been prepared for supermarket retailing and kale which was sourced directly from the farm, which had not been minimally processed by cutting, washing or vacuum packaging. Kale which was minimally processed contained 37%, 51%, 51%, and 47%, lower levels of L respectively, in the spring, summer, autumn and winter than freshly harvested intact whole kale sourced direct from the farm. Possible explanations for these variances are discussed below.

Fresh-cut vegetables and fruits are widespread throughout the fresh produce industry and refers to the process whereby raw vegetables and fruits are cut, shredded, peeled, abraded, or otherwise prepared to produce convenient ready-to-eat or ready-to-cook portions [264-266]. Additionally, this includes the final stage of packaging before being sold to supermarkets, restaurants, hotels and other smaller retail outlets. In earlier literature this process is also referred to as 'minimal processing' [267]. Consumer demand for fresh fruits and vegetables coupled with a demand for convenience is fuelling an interest in these minimally processed products. Since thermal and other drastic processing conditions are not used, minimally processed products are expected to retain fresh and be of superior nutritive quality. Low temperature and modified atmosphere packaging techniques (low O2, high CO2) are used which are intended to help retain freshness, extend shelf-life and limit the growth of micro-organisms [268]. However, studies to demonstrate the effects of minimal processing and modified atmosphere packaging on individual bioactive compounds, particularly in leafy green vegetables are still limited.

In this study, the L values of minimally processed kale were found to be significantly lower than that of freshly harvested kale ($p <0.001$) classified as being whole intact produce. Previous data which has investigated L concentrations in raw kale has only considered farmed kale or minimally processed kale separately [199, 202, 269], thus this is the first study to examine the L differences between raw intact kale and its corresponding fresh-cut kale commodities, grown from the same farm. The rapid deterioration in L concentrations of kale sourced from the marketplace may be attributed to several things. Firstly, fresh-cut vegetables deteriorate faster than intact produce, which may be a direct result of the wounding associated with processing, leading to a number of physiological and biochemical changes which differ from intact commodities [267, 270, 271]. Nutrient losses are generally accelerated following tissue wounding, which may allow for substrate–enzyme interactions. In addition,

exposure of inner plant components, including carotenoids and phytochemicals to oxygen during minimal processing enhances oxidative degradation [261].

The degradation in carotenoid concentrations may be further enhanced during kale shelf-life in the supermarket. In this study, L levels were found to decrease by 37-51% respectively, from farm to shelf throughout the year. Kale which is grown in the UK typically has a shelf life of five days; and is stored under continuous retail lightening at 8°C. Previous studies have found carotenoid concentrations in minimally processed kale to reduce dramatically at temperatures above 4°C, with or without light exposure. Kobori, et al (2011) found that at 11°C under light, L decreased in minimally processed kale by 7.1%, and 24.1%, respectively, after 5 days and 10 days on the shelf [272].

In terms of the seasonal or climatic effects on the carotenoid concentrations in kale, although statistically significant, this study found only limited changes in L concentrations in both kale varieties throughout various periods of the year. Minimally processed kale sourced from the shelf had higher variations in L concentrations through various seasons, where by L levels were significantly higher in the spring than any other time of year (M=13.40mg SD 0.004 $p<0.001$). In freshly harvested kale however, spring L levels were slightly lower than other seasons meaning the retention of L in minimally processed kale in spring may be related to post-harvest rather than pre-harvest climatic effects.

Two processes occur in photosynthetic tissues such as dark green leafy vegetables, which have opposing effects on carotenoid levels. These are known as caroteneogenesis and photo degradation. Exposure to sunlight and high temperature enhances the biosynthesis of carotenoids (caroteneogenesis), but also promotes photo degradation, which potentially lowers carotenoid concentrations. There is evidence to suggest that vegetables including kale produced in greenhouses, or in plots covered with plastic roofing, show higher carotenoid concentrations in the summer as opposed to the winter, as the plants are protected from excessive sunlight during the summer, thus favouring caroteneogenesis instead of photo degradation [199, 258, 273]. In the present work, kale which hadn't been subject to post-harvest cutting or packaging had highest L levels in winter, indicating that the effect of sunlight and high temperature on photo degradation prevailed in the spring, summer and autumn. The kale used in this study was farmed in open fields for the majority of its harvest life which gives added clarification to these findings. Two previous studies have also found carotenoid concentrations to be higher in the winter than the summer

for leafy green vegetables harvested in open fields, suggesting that sunlight and high temperature may promote photo-degradation when vegetables are not protected by roofing [202], [274].

3.6 Summary (effects of minimal processing and season)

This study adds to the small amount of information that is currently available concerning the retention of antioxidant constituents such as L in raw kale during handling and retail storage at different times of the year. These findings, as well as previous literature on other varieties of fruits and vegetables, indicate that fresh-cut produce has different properties to its corresponding intact vegetables, in that it has an increased perishability of nutrients. This means that the knowledge accumulated over many decades regarding the physiology and handling of commercial vegetables may have to be re-examined and new understanding and information developed for each fresh-cut produce item is needed. Further information and research is required on the chemical and biochemical alterations that occur in post-harvest minimally processed foods sold in supermarkets and on the improved maintenance of their nutritional quality, particularly for food sources that have not previously been addressed such as kale. In terms of climatic effects of caroteneogenesis, this study is in agreement with previous findings which suggest that carotenoid concentrations are higher in the winter than the summer for leafy green vegetables harvested in open fields, as sunlight and high temperatures may promote photo-degradation when vegetables are not protected by roofing or packaging.

3.7 Discussion (effects of cooking and storage)

The study described here explored the L levels in minimally processed retail prepared kale, and kale freshly harvested from the farm, following various domestic processing and storage procedures. Unlike fruits, most vegetables are commonly consumed after being submitted to a cooking process. Cooking methods are used to improve vegetables' palatability by softening the tissues, and inactivating anti-nutritional compounds, toxic substances and microorganisms. However, when comparing their biological actions and antioxidant activities in vitro and in humans, there is no consensus regarding the best way to consume them [204]. As a possible preventer of AMD, efforts to increase MP levels through simple dietary methods such as increasing dark green leafy vegetables are recommended. Therefore, gathering further information about the post-harvest effects on the L levels in kale is important, thus setting a precedent for this study.

Kale L levels within this study were dramatically affected by cooking and storage, with the notion that the longer the kale was cooked or stored the more L was lost. There were less apparent changes in L concentrations upon processing procedures which used shorter durations, lower temperatures and smaller concentrations of water such as blanching, liquidising and steaming. Possible explanations for these variances are discussed below.

Effect of cooking methods on the L concentrations in kale varieties

Steaming, boiling and stir-frying with oil:

Steaming, boiling and stir-frying are common domestic cooking methods in the UK, therefore these were the first processing techniques to be analysed in the initial pilot investigation in spring. As shown in tables 3.7 and 3.8, boiling for 3 and 5 min and stir frying with a small amount of olive oil resulted in significant losses of L in both varieties of kale. In both the shop and farm kale, the fried sample with oil showed the highest degradation of total L relative to the raw samples (83% shop, 88% farm, $p < 0.05$). Similarly, a recent study investigating certain phenolic compounds in kale after boiling, steaming and stir-frying, found that stir-fried kale (4 min with soybean oil) had the greatest decrease in L levels compared to all other cooking methods (4.66 ± 1.81 μg/g) [204]. Boiling for a longer duration of 5 min was more detrimental than boiling for 3 min in both kale types ($p < 0.05$) although both techniques resulted in substantial losses in L (boiling 3 min: 45% shop, 67% farm) (boiling 5 min: 69% shop, 89% farm). In both shop and farm kale, steaming showed the lowest degradation of total L relative to the raw samples amongst all processing techniques (14.8% shop, 46.6% farm) so would be the optimal cooking technique for retaining L levels in kale. In the study previously mentioned steaming kale was found to be more detrimental for L levels than boiling kale. Raw kale contained 56.18 ± 2.9 μg/g of L, after boiling and steaming this decreased to 6.9 ± 0.54 μg/g and 5.81 ± 1.14 μg/g respectively [204]. Other studies investigating steaming, boiling and stir-frying effects on L concentrations in kale are limited; therefore, further studies may be needed to support these findings.

Although freshly harvested raw kale was superior in L concentrations than minimally processed raw kale within its shelf life constituency, upon processing, L levels declined further in kale sourced from the farm. The reasons for this are likely to be related to the effects of minimal processing. Fat-soluble nutrients such as the

carotenoids (including L) are sensitive to heat, light, oxygen, and pH. Minimal processing, such as cutting, or slicing is useful from a convenience standpoint, however it causes injury to the plant tissues which initiates enzymatic changes, such as ethylene production, respiration, accumulation of secondary metabolites and water loss from tissues [275]. This increases susceptibility to microbial spoilage, which not only compromises food safety, but alters chemical make-up and promotes loss of nutrients [276]. Therefore, to preserve moisture and humidity as well as protect fresh-cut products, packaging is used. Packaging may help to preserve some nutrients in fresh-cut products, particularly if done at the right time and under appropriate conditions, mainly because it delays ripening and deterioration. Other techniques such as modification of pH, irradiation, or chemical preservation (dips in ascorbic acid and/or citric acid), a technique used in the kale sourced, may further prevent deterioration of minimally processed vegetables [197, 277]. It seems freshly harvested intact kale commodities may initially benefit from an enhanced nutrient value in their raw state, but exposure to heat and oxygen without the added protection of packaging and chemical preservation from acid washing may further increase their carotenoid degradation upon processing.

Frying without oil, microwaving, blanching and liquidising

After the pilot investigation in spring, kale was stir-fried for both 5 and 10 minutes without the use of oil. Previous studies investigating the use of stir-frying vegetables have found that frying treatments cause loss of (all-E)-isomers of carotenoids [204, 278], which could be explained by the lipophilic nature of carotenoids with the consequent leaching of these molecules into the oil. This may explain why L levels were lower in the pilot investigation where oil was used. Recent human intervention studies have shown that this leaching of carotenoids into oil may potentially improve their bioavailability, since the bioavailability of lutein has found to be higher when administered in oil [279-281]. Dietary fat increases carotenoid bioavailability by providing a depot for the release of hydrophobic compounds from the food matrix, stimulating the secretion of bile salts and pancreatic lipases for micelle formation, and inducing chylomicron synthesis [282]. A study which explored L bioavailability in kale found that approximately 5-10g fat in a meal was required for efficient absorption of carotenoids in to the blood, although more fat was required when the diet contained L esters instead of free L [283]. In the current study, dry fried kale samples without the use of oil still showed a high degradation of total L relative to the raw samples (59%-66% shop, 77-80% farm), with frying for a longer duration of 10 min being more

detrimental. Frying with the addition of oil in the pilot investigation resulted in significantly lower levels of total L than frying without oil (Mann-Whitney U test p =0.001), giving further evidence to support the lipophilic nature of these molecules.

Microwaved kale for the longest duration with the highest amount of water showed L degradation of 55% and 70% in shop and farm kale respectively, relative to the raw sample, suggesting that shorter processing times and the use of minimal water during microwaving kale would be preferred. To the best of my knowledge, this is the first study which has investigated various microwaving techniques on the L concentrations in kale. A small number of studies have investigated the effects of microwaving on other predominant carotenoid containing vegetables. The levels of carotenoids in raw and cooked (microwaved, boiled, steamed, stewed) green vegetables and tomatoes were extensively studied by Khachick et al, (1992). It was shown that while the epoxycarotenoids were somewhat sensitive to heat treatment, L and hydrocarbon carotenoids such as neurosporene, a- and P-carotene, lycopene, (-carotene, phytofluene, and phytoene) survived the heat treatments [284]. The effects of induction boiling, conventional boiling and microwave steaming on the sensory qualities and carotenoid retention of broccoli, carrots, green beans and sweet potatoes were investigated by Nunn et al [285]. The L/Z content of microwave-steamed broccoli was significantly higher ($p < 0.05$) than that prepared by either of the two boiling methods. The current study is in agreement with these findings, in that the concentration of L in microwaved kale using minimal water for 1 min (Micro 1) was significantly higher in both kale varieties than that prepared by boiling ($p <0.001$) or frying ($p <0.001$) for the shortest durations.

Blanched kale exhibited similar effects on L levels than steaming kale, showing a fair conservation of total L with a reduction of 15% and 50% for shop and farm kale respectively. Although intact whole kale sourced fresh from the farm showed a higher degradation than its minimally processed commodity, 100 g of blanched kale of this variety would still provide 10.85 mg of L. Similarly 100 g of shop purchased kale would provide a sufficient quantity of 9.83 mg. 10 mg of L per day has been associated with a delay in the progression of AMD [10] and the most beneficial effects on MP levels in the eye [98, 99]. The concentration of L in blanched kale of both varieties was not significantly different than that prepared by steaming (Mann Whitney U test $p = 0.92$), thus both methods may be considered as optimal cooking techniques for retaining L levels in kale.

Previous data in regard to blanching effects on kale are limited. One study which investigated total carotenoid losses in kale after blanching found that blanching kale leaves for 2.5 min did not significantly alter the level of total carotenoids and beta-carotene from raw [286]. Furthermore, a study which investigated the effect of blanching on Ethiopian and Indian green vegetables found that, blanching increased, in general, neoxanthin, L and beta-carotene contents to a slight degree, explaining their results with better extraction and quantification of these compounds from the denatured plant tissue [287]. Even if heat may result in some loss of carotenoids, the inactivation of oxidative enzymes by blanching may prevent higher losses of carotenoids during holding before thermal processing, slow processing and storage [288]. Akapap, et al. reported that blanching of fresh fluted pumpkin leaf, okra, African spinach, and water leaf at 98 °C for 3 min caused total losses of carotenoids which ranged from 29.5% in okra to 34.9% in both fluted pumpkin leaf and African spinach. It was further reported that blanching the vegetables before storage at -10 °C slightly improved the retention of nutrients, again because blanching possibility inactivated the enzymes [289].

Of all the processing methods, liquidising kale from raw showed the best conservation of total L in both kale varieties with a reduction of only 20% and 22% in shop and farm kale respectively. Although L losses were similar in the two kale varieties, the concentration of L in farm sourced kale after liquidising (M=9.31 mg/100g, SD= 0.12) was significantly higher than that of shop sourced kale (M=16.71mg/100g, SD= 0.10) (independent t test $p = <0.001$). Thus, it was evident that freshly harvested intact whole kale was less susceptible to damage by a processing method which did not require heat and therefore should be recognised as a superior source of L to minimally processed kale prepared for retailing, if this is the desired method of consumption.

Effect of storage methods on the lutein concentrations in kale varieties

As reported in the previous chapter, vegetables such as kale are often the most health-promoting when harvested and consumed at their peak maturity, before the addition of post-harvest handling and processing. In westernised countries like the UK, nowadays home-growing vegetables is outdated, and sourcing mass produced minimally processed products available freely in supermarkets is a more convenient, conventional consumer habit. Fruits and vegetables grown in the UK may spend up to 2 days in transit following harvest before arriving at a retail outlet. At the retail store, fruits and vegetables may spend a further 1–5 days on shelf display prior to being

purchased by the consumer, who then may store them for up to 7 days prior to consumption. This means that fresh fruits and vegetables may not be consumed for a significant length of time following harvest, during which time nutrient degradation may occur.

Due to continued respiration and enzymatic activity, fruits and vegetables suffer changes in nutritional value and sensory quality including loss of texture, appearance and flavour during storage [275], especially if factors such as temperature, atmosphere and relative humidity are not well regulated [277]. In the current study, it was found that fridge storage for the longest duration of 7 days showed a significantly higher ($p=<0.001$) L degradation (47% shop and 58% farm) than fridge storage for 4 days (62% shop, 76% farm), relative to the raw samples. The trend that the degradation of L levels increases further upon longer storage times is documented in other studies. Bunea, et al. reported that in spinach, the carotenoids such as L and beta-carotene were all affected by prolonged storage time and temperature. The content of carotenoids was best preserved after storage for one day at 4°C [259]. A study which investigated the effects of storage for four days at 7-9°C on the carotenoid contents of kale found that all four carotenoids decreased significantly; L (27%), violaxanthin (20%) neoxanthin (31%) and beta-carotene (14%). The authors noted that the retention of the carotenoids could have been improved, by using modified atmosphere packaging and a lower storage temperature [199]. Although storage temperatures were lower in the current study, the losses observed may have occurred due to longer storage durations and the use un-optimised packaging, as minimally processed kale was removed from its original packaging and both kale varieties were placed in simple sealable bags. Freezer storage also had detrimental effects on L concentrations in kale; however, L preservation was slightly better than that of fridge storage. Kale stored in the freezer for a longer duration of 28 days showed a high L degradation of 64% and 71% in shop and farm kale respectively, relative to the fresh samples, compared with 39% and 55% in freezer storage for seven days.

3.8 Summary (effects of cooking and storage)

Processing and storage of foods have become integral parts of the modern-day food chain. More emphasis should be given to increase consumer knowledge on the depletion of important nutrients in vegetables due to the post-harvest processing and storage techniques required for retail manufacturing or domestic processing.

Domestic cooking, storage and preparation have substantial negative effects on the content and bio-availability of carotenoids in foods. If domestic processing is to occur, reducing processing/storage time and using cooking methods which require lower processing temperatures and minimal amounts of water, improves L retention significantly, regardless of kale source.

Furthermore, it may be beneficial to suggest the method of steaming kale rather than boiling or stir-frying, and to advise more thoroughly on alternative methods to conventionally cooking kale, such as liquidising and blanching, or better still consuming kale raw. Although the use of oil may improve L bioavailability in the addition of frying, alternative cooking techniques which involve the addition of fat should be encouraged. Previous data suggests that blanching in particular may prevent subsequent nutrient losses if performed before storage due to the inactivation of certain enzymes. Blanched and pureed vegetables may result in increased extractability of carotenoids in vegetables compared to the raw homogenized form; however, there is paucity of information in this area. Loss of nutrients during fresh storage may be more substantial than consumers realize, so consumers should be educated about this. Fruits and vegetables should be consumed soon after harvest, or postharvest handling conditions must be controlled such that nutrient degradation does not occur. For example, shorter storage durations, exclusion of oxygen through modified atmosphere packaging, protection from light and low temperature diminishes carotenoid decomposition during storage.

In conclusion, L levels in kale which is both freshly cut and pre-prepared for retailing are negatively affected by processing and storage conditions. The evidence suggests that regardless of the method chosen, retention of L decreases with longer processing or storage time and higher processing temperatures. The results of the study described here can be used to enrich the current advice about kale consumption in those who suffer with AMD, and provided evidence to support the design and production of a novel intervention to improve diet in this population.

Limitations

These studies contain several limitations. Firstly, the processing and storage treatments used were not consistent throughout the seasons; for example, in the pilot analysis in spring alternative cooking methods were examined compared with analyses in following seasons. This procedure was adopted to address any methodological issues early on and to test various processing treatments in order to

alter and identify the optimum requirements for retention of L in kale. However, repeating the same analyses throughout every season would have provided more data for statistical comparisons. Secondly, temperature during frying was not measured and controlled which would have increased the reliability and repeatability of results. The kale types used in the studies were curly kale, though other varieties of kale are popular in the UK such as Cavalo Nero. It would have been useful to draw comparisons of L concentrations between varied kale types to provide further data on the optimum sources of L amongst dark green leafy vegetables grown and sourced in the UK. Overall, further research is needed to support the carotenoid composition of UK kale; comparisons with previous studies are difficult as variations are most likely due to differences in cultivars and environmental factors. Although the present data may provide new information to enrich the current advice about dietary L sources, there are many uncontrollable inter-individual variations that regulate the digestion, absorption, transport and eventual retinal uptake and maintenance of these nutrients [93, 104, 279, 290, 291]. It is therefore important for studies to not only assess the total L or carotenoid content of plants but also their stability along the food chain [292].

Chapter four will describe a qualitative study conducted to establish food choices and preferences in AMD patients, the physical and psychological factors which may influence eating habits and thus the potential barriers to dietary change. The acceptability of ready meals specifically designed to support the nutritional needs of AMD patients as a novel intervention for improving diet was also discussed with subjects to help inform the design of a questionnaire to use in a larger cohort of patients.

Chapter 4: Use of a focus group to identify eating habits in AMD patients

4.1: Background and rationale

Lutein and Z consumption can improve MP levels in the ocular media [293], which may be of benefit in the primary prevention of onset or progression of AMD. As L and Z are not formed within the body and can only be obtained from our diet their dietary intake is important in AMD patients [294], [81]. However, subjects who suffer with AMD are presented with a wealth of nutritional information available from a variety of sources such as magazines, newspapers and the internet. Conflicting information, a lack of evidential research and aggressive marketing campaigns have led to confusion among patients and practitioners in what supplements to take, and what foods should be consumed in order to maximise absorption of these useful nutrients [209].

Many patients turn to organisations for clarity of information such as the MS – the only UK charity that is devoted to helping those with diseases of the macula. The MS has 16,000 members, a busy helpline and counselling service and 235 local support groups nationally [210]. They produce training programmes and information for patients and health professionals. They also sponsor research into treatments, quality of life issues, a cure and campaigns for access to treatments and services [295]. Members of the MS may be considered an 'informed' population as the society provides patients with various educational tools to assist with increasing knowledge on nutrition and AMD. These consist of nutrition leaflets, articles in their monthly patient magazine 'Side View' and yearly health professional magazine 'Digest' all of which are updated to keep abreast of current research. Members and non-members also have access to a patient portal on the main website which includes detailed information on all areas of nutrition including diet and supplementation advice [296].

Despite this wealth of nutritional information presented by the MS, dietary analysis research within this population informs us that patients are not consuming enough nutrients that are recommended for their condition. A previous study conducted by our research group at Aston University and a follow up study addressed in chapter two have found that average L/Z intakes in this population were much lower than the recommended dosage for patients who suffer with AMD [211]. Participants also consumed daily calories intakes significantly below the government DRV's. Other important nutrients consumed below the DRV's included carbohydrates, fibre, and vitamin D and potassium.

Although these cohorts were groups of highly motivated individuals who had taken the initiative to contact the MS, the evidence does not suggest that the information that participants had received from the MS or other sources modified their behaviour [211]. There may be various reasons for this such as a dislike for the nutrient-rich foods, lack of knowledge on how to cook or prepare them, misjudgement on the amounts required per day, lack of control (family or caregivers cook food), and difficulty in acquiring or preparing foods. Although the association between education barriers in the visually impaired and diet-related health outcomes has not been studied in depth, some studies have considered the above factors [210, 251].

A study which assessed the perceived health and vision states and awareness of lifestyle factors of AMD patients looked further in to the barriers to why AMD patients who seek the services of the MS are under consuming nutrients, by determining their awareness of the relationship between nutrition and AMD. It documented that over half (56%) of AMD subjects were able to acquire food themselves, and used a supermarket as a primary source for acquiring food sources. However, the remaining 44% of patients were unable to shop for themselves. When considering patient's food choices, the largest influencer appeared to be preference (44%), with the next largest (33%) being foods that the subjects believe would affect their health. The ability to prepare the foods was a factor for 6% of subjects, and 3% said that the ability to acquire the food was the predominant influencer [297]. Further research from Douglas, et al's survey into independent living for the RNIB [251] showed that 'preparing a meal' was one of the most commonly identified challenging task by visually impaired people (33% respectively). Further information about how they acquired their food revealed that 56% of the participants were able to go food shopping; 28% relied on a family member, 3% had a friend and 2% had a caregiver who went food shopping for them. A further 12% of subjects had another method of acquiring food such as the internet, meals on wheels, etc.

Changing eating habits may be difficult in these older populated groups and requires novel intervention methods. Effective health education for promoting nutrition literacy among elderly people may only be the first step when encouraging dietary change. Current research suggests that there are numerous factors which may play a part in influencing AMD patient's dietary habits [210]. It is therefore essential to design effective measures for improving the diet of individuals with, or at risk of, AMD, which consider underlying barriers to dietary change.

The aim of this study was to design and conduct a focus group to discover what foods patients like to eat and in more detail, what factors may influence their food choice and preferences and the potential barriers to dietary change. The idea of ready meals as a potential intervention for improving diet in AMD patients was also discussed. The focus group method was selected for this study because it fostered open interaction and discussion among group members which generated rich data related to the individual needs of older adults living with visual impairment.

Ethics

This study was approved by the Aston University Ethics Committee (Ethics number 843). Verbal informed consent was obtained from all subjects and formally recorded. Verbal informed consent was obtained from all subjects and formally recorded.

4.2: Method

Step by step scheme of work

1. Recruitment of AMD patients for focus group via a Macular Society local peer support group meeting
2. Carry out focus group with AMD patients
3. Design questionnaire for larger cohort based on findings from the focus group
4. Carry out questionnaire in larger cohort
5. Design ready meals for AMD patients based on the findings from the focus group and questionnaires.

Subjects of focus group

Patients of the MS who are members of a local peer support group were invited to take part in a focus group to discuss their individual food choice and preferences and possible physical and psychological barriers to dietary change. Six AMD patients were invited to join the focus group. Inclusion criteria were subjects with either form of AMD who were over 50 years of age.

Method

One focus group was conducted involving six AMD patients. The session was held at a local peer support group for MS members, where participants were recruited from the peer support group beforehand. The peer support group involves forty-five MS members. Subjects who agreed to take part in the focus group were asked to stay after their peer support group meeting to be involved in the 90-minute session. The

focus group was conducted by a facilitator and assisted by a moderator who took detailed notes throughout the discussion. The focus group was also transcribed by the moderator where possible. Before the discussion session began, the facilitator and moderator introduced themselves to the participants and started the ice-breaking session in which each of the participants were asked to briefly introduce themselves to the group. The session then proceeded with an introduction to the study where the aims and objectives were explained and the confidentiality of the outcome from the focus group. After the introduction, the discussion session was initiated by asking semi-structured and open-ended questions to subjects. The semi-structured question design was centred on the following topics: the way that participants choose and prepare their daily foods, the factors influencing their food choices and eating habits and possible barriers to not making appropriate dietary changes. This included questions such as:

Which foods do you like to eat/ what is your favourite food?

Why do you like the food?

What foods do you not like to eat?

Why do you not like the food?

Do you eat more hot or cold foods?

What meals would you normally prepare?

Do you often make meals with dark green leafy vegetables such as kale?

How do you usually like to cook these foods?

If not, why do you not include these foods in your meals?

How do you feel about ready meals?

How do you feel about cooking home-made meals?

Where do you normally shop?

How much do you like to pay for an average dinner (ready meal/home cooked)?

How much would you spend on an average ready meal?

The sample consisted of one male and five females who were Caucasian and aged above 50 years. All participants presented with some form of AMD. The themes that

emerged from the data focused on the food/meal likes and dislikes of patients, patient cooking habits, patient's opinion on ready meals, and perceived barriers of dietary change.

Analysis of focus group

Data from the focus group was analysed using a Micro-interlocutor Analysis, which is a relatively new method of analysis for focus group research, taken from an article by Onwuegbuzie, et al (2009) [298]. Micro-interlocutor analysis goes beyond analysing only the verbal communication of focus group participants and therefore increases the rigor of focus group analyses in qualitative research. It may be described as a process where meticulous information about which participant responds to each question, the order in which each participant responds, response characteristics and the nonverbal communication used is collected, analysed, and interpreted. A template which allowed for the transcribing of this information was used to analyse and interpret data (appendix 2). Themes that emerged from the focus group discussion were identified and are highlighted below.

4.3 Results

Food/meal likes and dislikes of patients

All participants agreed that fish was the preferred choice of food to base around a meal, with fish pie or fish accompanied by sides being mentioned as participants' favourite food/meal. One subject revealed that this was primarily white fish. Other favourite foods or meals consisted of lasagne (participant 1), chicken risotto, soup (participant 2), stir fry (participant 3), soup (participant 4), chilli con carne, quiche, curry (participant 5) chicken risotto (participant 6). Some of the reasons behind subjects' food preferences included taste, texture and digestibility. Food/meal dislikes included tuna, pilchards (participant 1), and spicy food (participant 2-4). Two participants indicated that they did not dislike any particular foods. Spicy food was specified as causing issues with digestibility, and oily fish was considered to be unsavoury.

Patient cooking/eating habits

All six participants enjoyed both hot and cold meals. Cold meals mentioned by all participants as being particularly favourable included quiche, salads and cold fish. Only two out of six subjects home cooked their meals on most days of the week. One subject lived with a relative who regularly cooked for them. The remaining three

subjects cooked two times a week, and on the other days of the week ate ready meals – this included supermarket purchased meals and home delivered meals such as Wiltshire Farm Foods (www.wiltshirefarmfoods.com). All three of these patients lived alone and mentioned this as being one of the main reasons for not cooking as often.

The three participants who were most likely to home cook meals frequently included dark green leafy vegetables such as kale in their dishes. Kale was predominantly used as a side dish in a meal. Two of these subjects stated that they mostly enjoyed stir frying kale with other vegetables. The three subjects who cooked less home-made meals rarely added kale to their meals, despite them being aware it contained nutrients important for their condition. This was mainly due to a lack of accessibility, issues with food preparation and a dislike for food wastage. All participants were unsure of ideal methods in which to prepare, store and cook kale for the retention of important nutrients, but were particularly interested in knowing so. Two subjects identified this lack of information to be an additional factor for not including kale in the diet.

Participant's opinion on ready meals

Two out of six subjects did not consume ready meals due to a perceived unpleasant taste, a concern with the addition of additives and preservatives and high fat and sugar contents. They also mentioned that they enjoy the concept of acquiring food and cooking. These participants disclosed that if they were to consider ready meals in the future they would have to be unrepresentative of the usual supermarket convenience food that they deem as 'unhealthy'. Other subjects enjoyed the idea of ready meals because of the convenience and simplicity that they offer. One point that was frequently mentioned by this group was the dislike for fresh foods going to waste when they are only acquiring and cooking food for themselves. Subjects further highlighted that acquiring, preparing and cooking foods were particularly difficult for them without the added support of another person. When buying vegetables, these subjects tend to choose frozen alternatives. All participants would not let price influence their choice over a ready meal, with nutritional impact and taste being the two key drivers for choice. All subjects agreed that if they were to be influenced by price then between £2 and £4 or even above £4 would be reasonable.

Perceived barriers of dietary modification

Food wastage and storage concerns. 'Kale comes in a large 200g bag with much of it going to waste as I am only cooking for myself. This puts me off buying it despite

knowing it is good for my eyes', stated one participant. Other patients agreed with this statement and said they would prefer to have access to more local independent suppliers of kale where they could choose their own amounts of produce. For example, one participant commented that 'kale I buy from the supermarket tends to go off very quickly when it is not used, I would like to be able to buy kale from local producers where it is fresh and not pre-cut and packaged'. Another participant added 'I would like to know if I could freeze the excess kale and then re-use it or would this have an effect on its nutrients'. Most patients agreed with this query, two patients stated that they prefer to purchase frozen vegetables as they last longer.

Acquiring, preparing and cooking foods. Only two out of six patients experienced cooking their own meals on regular days of the week. One patient revealed 'I live with my son who cooks and prepares food for me; otherwise I would struggle to be independent'. The remaining patients agreed with this statement, claiming that health, mobility and visual impairment prevent them for acquiring, preparing and cooking foods as much as they would like to. 'When you are on your own you do not want to prepare, and cook for one person, it is too much effort and food goes to waste so I prefer to eat cold meals or heat up ready meals in the microwave' said one elderly man. Patients who lived on their own were in agreement with this statement, preferring to opt for foods requiring limited preparation or cooking. 'I use Wiltshire Farm Foods to deliver my ready meals for the same reasons, however I have not come across any ready meals they do which contain kale, that would be good' another woman stated. Patients that incorporated kale in to their meals had various opinions and ideas on cooking methods. Two patients stated 'stir fried kale is the tastiest and you can add all sorts of other vegetables, oils or sauces' another remarked 'is this a good way to cook kale?' One patient had opposing opinions stating 'I prefer to steam my vegetables I have heard this method is best for retaining nutrition and keeping flavour and texture'.

4.4: Discussion

Research has shown that AMD patients are not taking on board the recommendation dietary advice for their condition [211]. The reasons for this are likely to be multi-factorial. In a previous study within a similar cohort of patients, participants were asked through the use of a survey what influenced or dictated their choice of food. The largest influencer appeared to be preference (44%), with the next largest (33%) being foods that the subject's believe would affect their health. The ability to prepare the foods was a factor for 6% of subjects, and 3% said that the ability to acquire the

food was the predominant influencer [200]. Results from the focus group present similar findings, a person's food choices and preferences are very much individually varied. Within this small cohort of AMD patient's individual constraints to not consuming a diet recommended to support the nutritional needs of their condition were difficulty with acquiring, preparing and cooking foods, a dislike towards food wastage and a lack of information regarding optimal cooking and storage methods for vegetables such as kale. These barriers were further emphasised in those who lived alone and lacked the support of family members or carers. Strategies to ensure that nutritional information and interventions consider these multi-factorial barriers within this vulnerable population may be needed.

Only two out of six patients experienced cooking their own meals on regular days, acquiring and preparing food was noted as being an enjoyable factor in this. One patient was solely dependent on a relative to do this for them and the remaining subjects (half the cohort) opted for convenience food such as ready meals or meals that required minimum preparation. Difficultly acquiring preparing and cooking foods when living alone led to more interest in these food choices. Living alone can have a tremendous impact on quality of life - The RNIB's 2003 commissioned survey of 588 BT customers found that 63% of blind and partially sighted people live alone, which is a much higher figure than the elderly population without sight impairment [15]. Participants were aware that dark green leafy vegetables contained important nutrients to support eye health, however only half of the group incorporated kale in to their meals on a regular basis. Food wastage was a concern with all patients who were in agreement that the proportions of pre-packaged kale available in most supermarkets were too large. There was significant confusion amongst subjects on the best ways to cook and store kale and all patients anticipated further information within this area. Frozen foods such as vegetables were particularly appealing to those who were living in a single occupancy household and seeking convenience from their meals.

Ready meals were a primary attribution to the diet of half of the patients within this group. Participants who live alone and find it difficult to acquire, cook and prepare foods benefit from the simplicity and convenience that these meals offer. However, two out of six subjects had an aversion towards ready meals due to the 'unhealthy' connotation that surrounds them. Subjects were concerned with the notion that ready meals contain additives and preservatives and likely high sugar and fat contents. Subjects were also keen to gain added information about how they should cook, prepare or store foods recommended for their condition, such as kale. Further

information in this area may give patients more practical advice for encouraging dietary change.

Limitations

One criticism of qualitative focus group analysis is that findings are usually not generalisable beyond the local research participants as it typically involves examination of data extracted from small, non-random samples [299, 300]. The rationale for the small focus group size within this study stems from the goal that focus groups should include enough participants to yield diversity in data provided, yet they should not include too many participants because large groups can create an environment where subjects do not feel comfortable sharing their thoughts, opinions, beliefs, and experiences [301]. Krueger (1994) has endorsed the use of very small focus groups, what he terms “mini-focus groups” where participants have specialised knowledge and/or experiences to discuss in the group [302].

A distinct limitation to the current study may be the use of only one focus group to collect data. The number of times a focus group meets can vary from a single meeting to multiple meetings however, using multiple focus groups allows the assessment of the extent to which saturation has been reached [303], [304]. Data saturation may result when information occurs so repeatedly that the researcher can anticipate it and whereby the collection of more data appears to have no additional interpretive worth; or theoretical saturation when the researcher can assume that the emergent theory is adequately developed to fit any future data collected [305]. The subjects used in the current study are, of course, not a truly accurate representation of all patients seeking services from the MS; however a more diverse group with a range of cultures and socioeconomic levels would likely raise a range of viewpoints. This research also solely focuses on AMD patients who are members of the UK charity the Macular Society. It would also be important to find out the opinions of those with AMD who have not sought support from non-professional organisations.

4.5: Summary

Ageing is characterized by a decrease in activity [306], a decline in lean body mass [307], and loss of appetite [308] which may lead to poor dietary impacts in older adults. This study indicates, however, that barriers to eating sufficient energy requirements and following a healthy diet may include both the consequences of normal ageing for the control of appetite, and changes in psychosocial circumstances which may

exacerbate diminished appetite such as fewer social eating occasions, living alone, and poor cooking skills. These barriers may be heightened in subjects who suffer with sight loss who have additional physical barriers involving food preparation and eating. It is evident that subjects recognise the importance of following a diet which contains important nutrients to help slow the progression of AMD, yet altering dietary habits is proven difficult when there are underlying individual barriers to change. Ready meals which are nutritionally tailored to support the needs of these individuals may be a promising intervention for improving the diet of AMD patients in the future. The results of this study will inform the design of a questionnaire to use in a larger cohort of subjects from the Macular Society to further determine AMD patient's interest in the design and production of such meals.

Chapter seven will describe the results of a survey which was designed to establish AMD patient's opinions and current usage of ready meals, their food likes and dislikes and their future interest in ready meals nutritionally tailored to support the needs of AMD. The survey generated rich data which aided with the design and production of these meals.

Chapter 5: Use of a survey to establish AMD patient's usage and opinions on ready meals

5.1: Background and rationale

It is evident that convenience plays a prominent role in the food choices of today's consumers. Demographic changes have produced an increasing demand for food products which are regarded by many consumers as convenient and affordable [309]. Rapidly growing Westernised populations typically consume fewer home cooked meals and more pre-prepared food products, such as ready meals and fast food. The convenience food sector is expanding rapidly; currently the US and the UK are the largest ready meal markets in the world, respectively valued at £7.2 billion [310] and £2 billion [311]. In Western Europe, the size of the market is estimated at £3.9 billion [312]. The majority of this is due to the UK market, which is expected to grow by 20% by 2017 [313]. Convenience food now constitutes more than a third of the British food market with approximately 8.8 kg of chilled and frozen ready-made meals consumed per capita per year [314]. This makes UK citizens the largest consumer of ready-made meals in Europe and the second largest worldwide (after the US); they are also the largest consumers of chilled ready-made meals in the world [313].

This growth in demand for 'convenience' foods that requires little preparation can be attributed to the population rise, increase in life expectancy, increase in single occupant households, lack of cooking skills and the changing needs of modern households including the shifting routines and rhythms associated with increased female labour-force participation [315], [316]. These foods represent a quick and easy alternative to home-prepared meals, as they are sold in a part or completely cooked form and are ready to eat within minutes, requiring no further ingredients or preparation other than heating [317]. Before reaching the consumer, ready-made meals already undergo some form of processing to ensure food safety or hygiene or to enhance palatability, texture or flavour. Processing may involve the addition of other foods or ingredients, such as preservatives, as well as heating, cooling or pressure-cooking. The distinction from raw or unprocessed foods or meals prepared at home is that the consumer cannot control the nutritional quality of the basic ingredients; therefore convenience foods are associated with palatable but less healthy food options. This has given rise to serious concerns about public health, as it has been argued that the consumption of convenience foods such as ready meals are linked with the consumption of more energy, saturated fat, salt and fewer fruits and vegetables [318], [319].

In practice, however, the ‘convenience’ food sector is extremely diverse with a wide array of foods in frozen, chilled and other formats. More than 90% of ready-meals sold in the UK are supermarket own-brand products [320]. Most supermarkets ‘brand’ their own-brand products into premium or luxury, ‘healthier’, economy or value, and standard ranges [321]. Fresh and frozen varieties of many ready meals are available across these ranges. As well as supermarket sold ready meals, there are also many food production companies which offer home delivered ready meal services. Examples include Wiltshire Farm Foods, [322], Oakhouse Foods [323] and Lodge Farm Kitchens [324]. These home delivered ready meal companies range from large franchises to small family run businesses, so ready meals are expected to range in quality, nutritional value and price.

As ready meals are often criticised for being ‘unhealthy’, the UK government’s Change4Life initiative [325] advises against frequent consumption of ready meals, as these food products, and diets high in these, tend to be more energy dense and higher in fat, saturated fat, salt and sugar, and lower in fibre than is optimal for health [326], [327], [328]. However, few studies have comprehensively examined the nutritional content of ready meals sold in the UK market. Studies that have are focused solely on ready meals purchased from supermarkets and are therefore not representative of the entire market. A study which investigated the nutritional quality of one hundred own brand ready meals sold by three leading UK supermarket chains found them to be high in protein, fat, saturated fat, and sodium, yet low in carbohydrates, and within the recommended range for sugar according to the World Health Organisation nutritional guidelines for the avoidance of diet related diseases [329]. Similarly, a recent study which investigated the nutrient and cost profile of 166 ready-meals from forty-one UK stores found that overall; ready-meals were high in saturated fat and salt, and low in sugar. One-fifth of meals were low in fat, saturated fat, salt and sugar, including two-thirds of the ‘healthier’ option ready meals [330]. Interestingly, a study which compared the nutritional properties and cost of the ten most frequently purchased ready meals in a sample of Scottish households to equivalent meals cooked from fresh ingredients found that in the ten most frequently purchased meals (pizza, chicken curry, lasagne, macaroni cheese, cottage/shepherd’s pie, Chinese chicken dishes, fish pie, spaghetti bolognaise, pasta and chicken bake and jacket potatoes) there were no significant differences between the ready meals and home cooked recipes for energy, macronutrients, fibre or sodium [331].

There has been a strong need to assess salt in ready meals, hence the development of these 'healthier' alternatives. The combination of food reformulation with improved food labelling and initiatives to raise consumer awareness on unhealthy foods has already led to successful national-wide salt reduction programmes, e.g. in Finland and the UK [332], [333]. Although there are commercially available 'healthier' alternatives in the ready meal industry, problems associated with nutritional imbalance of these meals is still apparent. Since the importance of convenient ready meals is likely to persist, different ways of addressing the problem of nutritionally unbalanced meals should be encouraged. Creating and offering consumers more ready meals with appropriate energy content, balanced macronutrient distribution and optimisation of nutritional value (such as the addition of vegetables), may have a better impact on consumer health.

The relationship, existing between adequate health and fresh fruit and vegetable consumption has been clearly documented, where regular consumption is associated with the prevention of chronic diseases such as heart disease, cancer, and diabetes [334-336]. The UK Department of Health have established a "five a day" programme to improve access to, and increase consumption of, fruit and vegetables [337]. In terms of AMD, there is evidence to suggest that increasing intake of certain nutrients found in vegetables may be helpful in delaying the onset or progression of macular degeneration [8, 10]. In the AREDS 2 trial 10mg of L per day was associated with the most beneficial results in AMD patients [10, 98]. Other studies have suggested that doses of 10mg/day of L or higher are associated with the most positive effects on MP levels [98, 99]. In dietary terms this would equate to a 50-100 g serving of uncooked kale per day (chapter four), depending on kale source. Despite this, AMD patients who seek the services of the Macular Society are not taking on board the dietary advice and recommendations related to AMD, which may in fact require novel interventions [210, 211]. Results from the focus group suggest that educational tools to improve patient's knowledge on aspects of diet and AMD have so far been unsuccessful; therefore, a more practical approach may be necessary.

It is clear that convenience has a significant impact on the food choices of today's consumers which may be particularly apparent in those suffering with sight conditions that tend to have difficulty with acquiring and preparing foods. This suggests that food products which offer more convenience may be deemed more preferable to these consumers. Therefore, adding convenience traits to certain products considered

healthy and/or beneficial, such as fresh fruits and vegetables, could increase the consumption of these specific food items.

The aim of this research was to investigate the current usage and opinions of ready meals in AMD patients and the potential interest in ready meals nutritionally tailored to support the needs of their condition. Creating ready meals which contain important food sources for AMD, which do not possess the 'unhealthy' traits of conventional supermarket ready meals and are nutritionally balanced, may lead to an increased consumption of these foods, and consequently, better ocular health for AMD patients.

Ethics

This study was approved by the Aston University Ethics Committee (ethics number 843). Verbal informed consent was obtained from all subjects and formally recorded.

5.2 Method

A literature search was unable to provide a similar survey to use as a template. Therefore, a cross sectional survey was designed with both open and closed ended questions which were created with the help of the outcomes of the focus group. The survey was then piloted over the telephone on six AMD patients from the MS sight impairment register who volunteered to take part. These volunteers were asked to then validate the questionnaire at three weeks later. Each volunteer had an informal discussion with a moderator – the survey questions were asked again to check if the answers matched, and then the volunteers were asked to comment on how easy the questions were to understand and opinions on the topics covered. The telephone pilots were recorded and analysed to further refine the survey. The survey was then administered to the cohort.

The cohort consisted of patients on the MS sight impairment register, members of a local MS peer support group and MS members who participated in the previous dietary analysis study (chapter 2). The initial questions covered demographic topics such as age, gender and type of AMD. The following questions focused on the current usage and opinions of ready meals, the foods they like to eat (in ready meals or normal meals) and the potential interest in ready meals that were nutritionally tailored to support the needs of their condition (questionnaire attached in appendix).

Inclusion criteria

The inclusion criteria included those aged over fifty-five who had been diagnosed with a form of AMD; the exclusion criteria were the inability to hear and reply to questions

in English over the telephone. There was no need to define and categorise the amount of visual loss the participant had, as the objective was to assess typical patients seeking the MS services.

Recruitment of subjects (MS sight impairment register)

People who were registered on the MS impairment list who suffered with some form of AMD were invited by letter to complete the survey. The letter explained the study procedure and protocol including the inclusion and exclusion criteria for those wishing to be involved (attached in appendix). The letter included a telephone number and email address that they could use to contact the investigator if they wished to participate in the study. They were advised that the investigator would call them back at a convenient time for them to conduct the short questionnaire. Before the telephone survey began participants were made aware that the results of the questionnaire were not to be used other than to aid the design of ready meals nutritionally tailored to support the needs of AMD patients.

Recruitment of subjects (MS local peer support group)

Members of the MS who are members of a local peer support group were invited to take part in the survey via email by their group leader. The peer support group involves forty-five MS members. Like the letter, the email explained the study procedure and protocol including the inclusion and exclusion criteria if they were interested in participating. Patients were advised that if they wanted to take part in the survey then they could contact the researcher by telephone or email to confirm their interest. They were advised that the researcher would call them back at a convenient time for them to conduct the short questionnaire. Alternatively, subjects were given the option to fill out the questionnaire themselves at the next group meeting.

Recruitment of subjects (MS patients who were previously involved in the dietary assessment study)

Members of the MS who were involved in the previous dietary analysis study were invited to take part in the survey over the telephone. Subjects were advised of the study procedure and protocol including the inclusion criteria before they gave their verbal consent. If subjects were happy to participate and met the inclusion criteria, then the questionnaire was conducted. Before the telephone survey began participants were made aware that the results of the questionnaire were not to be

used other than to aid the design of ready meals nutritionally tailored to support the needs of AMD patients.

5.3 Results

Table 3.6 displays the demographics of the included subjects. In total, there were fifty AMD participants aged 56-83 (mean 72 ± sd 6 years). Of the cohort, 36% were male and 64% were female. Dry AMD was more predominant within the cohort with 64% of subjects diagnosed with this form, compared with 36% of subjects diagnosed with wet AMD.

Characteristic	Characteristic	Percentage of AMD patients
Gender	Male	36%
	Female	64%
AMD type	Wet	36%
	Dry	64%

Table 3.6 Participant characteristics.

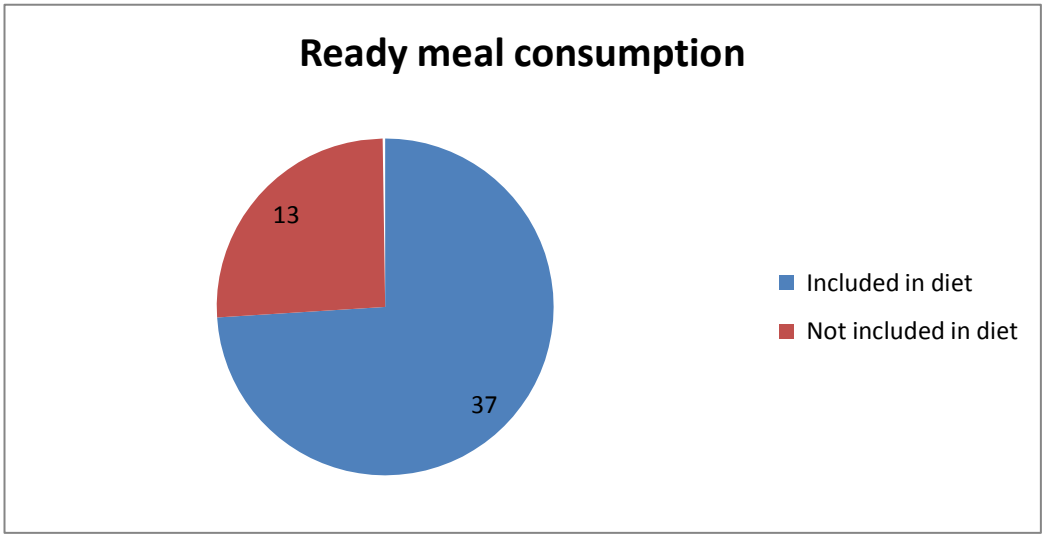

Figure 3.4: Ready meal consumption in participants.

Subjects current ready meal usage

The first and most important question on the survey was whether or not subjects consumed ready meals currently. Thirteen out of a total of a total of fifty subjects did not include ready meals in their diet and thirty-seven subjects did (figure 3.4). Subjects who did not include ready meals in their diet were referred to later questions

in the survey which were designed to find out if they would consider including ready meals in their diet which contained kale and which were nutritionally tailored to support the needs of their condition. Of the thirteen subjects who did not consume ready meals, eleven would consider ready meals which contained kale. One subject who opposed this idea mentioned that they take the medication 'warfarin' on a regular basis so avoid large amounts of green leafy vegetables due to the high content of vitamin K. Twelve of these subjects would however consider ready meals in their future diet which were nutritionally tailored to support the needs of their condition.

The main reasons for not consuming ready meals amongst subjects were having a spouse or relative who regularly cooks for them, a dislike towards their taste and a disapproval of the 'unhealthy' conception which is associated with processed foods. One subject revealed that they suffer with various allergies so avoid including pre-prepared foods in their diet. Convenience was the key driver for inclusion in the diet, as the majority of those who included them listed in the open ended comment box. Other reasons cited for inclusion in the diet included living alone, difficulty cooking, dislike towards cooking and the general enjoyment of the taste of ready meals.

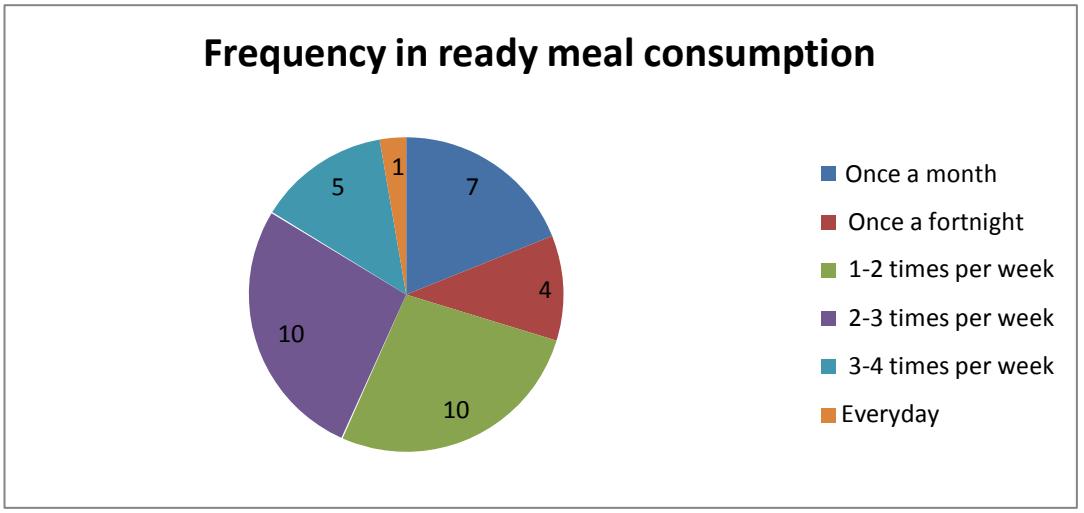

Figure 3.5: Frequency in consumption of ready meals for subjects who included them in their diet.

In regard to the frequency in consumption of ready meals, seven subjects consumed them once a month; four subjects consumed them once a fortnight, ten subjects consumed them 1-2 times per week, ten subjects consumed them 2-3 times per week, five subjects consumed them 3-4 times per week and one subject consumed them every day.

Cost and source of ready meals in subjects

Twenty-seven out of the thirty-seven subjects who consumed ready meals would be happy to pay over £4 for them. A total of eight subjects would prefer to pay between £2 and £4 and one subject would prefer to pay under £2. All subjects used a supermarket as the main source for acquiring their ready meals; however, five of these subjects also used an online distributor to purchase meals over the phone or via the corresponding website such as Oakhouse Foods (www.oakhousefoods.co.uk) and Wiltshire Farm Foods (wiltshirefarmfoods.com).

Consideration of ready meals which contained kale and which were nutritionally tailored to support the needs of AMD

All subjects who took part in the survey, including those who currently do not consume ready meals, were asked if they would consider ready meals which contained kale and ready meals that was tailored to support the needs of people with AMD. Out of a possible fifty subjects, forty-seven would consider consuming ready meals which contained kale. As previously mentioned one of the subjects in disagreement with consuming kale who did not include ready meals in their current diet avoided dark green leafy vegetables due to the high content of vitamin K which can have adverse interactions with the medication Warfarin. Despite this, forty-eight subjects would consider ready meals which were nutritionally tailored to support the needs of their condition.

Food likes and dislikes

Fish, chicken, beef, lamb and pork were popular with all subjects in the cohort. Nine subjects mentioned that they avoid frequent consumption of red meat so prefer fish and chicken alternatives on a daily basis. There were no subjects who only consumed vegetarian food options however most subjects did enjoy them. Some subjects mentioned that they like a variety of fish and meat in their diet. Only fifteen out of the total number of participants liked foods which you can eat without utensils for example pasties and pies. Some of the feedback received during the survey about these food items were that patients deem them as ‘unhealthy’. The majority of subjects did however enjoy eating one pot meals such as soups and casseroles (48 out of 50 subjects ticked this box). Extra feedback drawn out from the survey revealed that vegetable soups in particular were favourable with subjects. Some subjects cited that they enjoy casserole dishes but do not cook them often due to long cooing durations and the nature of the dish being large in size when home prepared, which inevitably

leads to food wastage in those who live alone. Popular examples of what foods subjects like to eat in ready meals or normal meals included soup, casseroles, fish pie, pasta dishes, cottage pie, curry, and lasagne. The majority of those who gave pasta as an example of a favourite or popular dish in their diet indicated that they prefer a creamy sauce base to accompany it.

Foods or ingredients subjects find difficulty with due to sight impairment

The final question on the ready meal survey was designed to encourage patients to give examples of any foods or ingredients that they may find difficulties with in their diet due to being visually impaired. Thirty out of a total of fifty subjects did not have any difficulties or aversions to certain foods. Of the remaining subjects, six indicated that they did not like meat or fish with bones due to finding it problematic when recognising them with impaired vision. Two subjects highlighted an important issue in regard to meals being too mixed together, for instance in vegetarian meals or stir-fries where there is an array of ingredients which are finely chopped together. This makes it difficult for those with sight impairment to differentiate between the various items in the meal and therefore be aware of what they are consuming. Other subjects had individual aversions to certain food items such as liver and offal, starchy and spicy foods.

5.4 Discussion

Within this cohort of fifty AMD subjects who are members of the MS, a total of thirty-seven included ready meals in their diet. The majority of these subjects (70%) consumed these meals at least once per week however 19% of subjects consumed them infrequently (once a month). Although recent population-representative data in the UK on the frequency of consumption of ready meals is not available, in 2006 it was estimated that around 40% of UK households ate such meals at least once per week. Convenience was a major influence behind subject's desire to include ready meals in their weekly diet, this was further emphasised in those who lived alone and lacked the support of family members or carers. Twenty-six out of a possible thirty-seven subjects lived alone and enjoyed the ease and simplicity that ready-made meals have to offer. In the focus group discussion with MS members in the previous chapter, four out of six members reported living alone to be a key barrier to achieving a healthy diet. This is similar to the finding of the RNIB's 2003 commissioned survey of 588 BT customers [15] who ordered their bills in large print or braille, to examine their living arrangements and quality of life. It documented that 63% of blind and partially sighted people live alone – a higher figure than the 'normal' sighted elderly

population (32%). In further discussions subjects gave details about the difficulty and inconvenience of preparing and cooking home cooked meals for one, and their concerns with food wastage when regularly purchasing fresh fruit and vegetables. In light of these findings, it may be proposed that frozen ready meals which require no addition of added ingredients, which are easy to prepare and available in portion sizes adequate for a single consumer, would be a suitable alternative to a home cooked meal in those living with sight impairment.

Despite the majority of subjects enjoying the concept of ready meals in their current diet, there were a small number of subjects who opposed this idea. Popular reasons for this included a concern with the negative connotations which surround such meals, a dislike towards their taste or having a spouse or relative that regularly cooks for them. These findings are not surprising given the fact that ready meals are pre-prepared and the consumer cannot control the nutritional quality of the basic ingredients or the amount of added salt, saturated fats or additives and preservatives used. Inevitably, this leads to a belief that such foods contain unfavourable amounts of these nutrients. A survey which collected nutritional data on 166 supermarket own-brand chilled and frozen ready-meals available in branches of ten national supermarket chains in one city in northern England, found that the nutritional content of ready meals varied substantially according to meal range and type [338]. Overall, meals were categorised as high in saturated fat and salt, and low in sugar according to current UK guidance for front-of-pack nutritional labelling [339]. This may indicate that maximum nutritional benefit is more likely to be derived from home cooking of nutritionally balanced recipes primarily using raw ingredients, rather than relying on manufactured ready meals which are created on a larger scale [340]. Other previous work which has attempted to compare the absolute nutritional content of ready-meals with nutritional standards for meals found that ready-meals often contain substantially fewer calories than is recommended for a meal [341], [342]. Ready-meals are perhaps more sensibly considered ‘ready-main-courses’ than complete meals, which may explain why the total energy content is less than might be expected. This is an important concept for this research as within previous dietary analysis data (chapter 2 and [211]) it has been revealed that AMD patients who seek the services of the MS are consuming calorie intakes which are significantly below the government DRV’s. As such, meals ought to be designed that are not calorie dense, yet still of high nutritional quality and which incorporate a tasty, home-cooking style method using freshly sourced ingredients. This may increase the desire for these products in this population and also help consumers to achieve a more balanced diet.

In total forty-eight subjects, would be interested in the idea of consuming ready meals that were nutritionally tailored to support the needs of AMD by including ingredients which are high in L and Z. When asked how much participants would be willing to pay for a ready meal, over half responded that they would pay more than £4 per meal, with the remaining participants willing to pay less than this amount. This is a promising finding, despite a total number of thirteen subjects who did not consume ready meals currently. It seems patients are keen to improve their diet, and ready meals which contain adequate levels of L and Z through the supply of dark green leafy vegetables may be a feasible way to do so. Popular examples of favourite meals included fish pie, creamy pasta dishes, cottage pie, curry, and lasagne. Soups and casseroles were also extremely popular dishes within this cohort, with many subjects revealing that they do not cook them due to being large dishes which require lots of time and preparation. This may be related to the large number of subjects who live alone, as those that live alone are much more likely to find their visual impairment a hindrance than those that live with other people. Perissinotto *et al's* study into the functional decline of the elderly (2012) found that loneliness was strongly associated with functional decline; those who reported being lonely were more likely to have a decline in activities of daily living, develop difficulties with 'upper body tasks', experience a decline in mobility, and have difficulty in climbing stairs [343].

Certain concerns for those who are severely sight impaired must be taken in to consideration when creating ready meals for this population. For example, a small number of subjects had issues recognizing bones in foods, or differentiating between various ingredients in a meal when they are too finely chopped or mixed together.

Limitations

Although the depth of detail provided from both open and closed ended surveys provides strength to the methodology and suggests reliability of results, there are obvious limitations in this study. Due to time constraints this was a small study, with a relatively small sample of AMD subjects who were all members of the MS. Patients who have sought the help of the MS could be considered an 'informed' population as they have information available to them in the form of monthly magazines, written material, a helpline and the Society's website. Since the AMD cohort all had a form of AMD, and were motivated to contact the MS for assistance, it may be presumed that they were engaged and proactively interested in preventing the disease from progressing. Therefore the results may not be generalisable to other populations, such as those with AMD who may be managing alone, without the society's support.

Another limitation to the study was the lack of control or comparator group. Comparing data to a cohort of age matched subjects without the condition would have provided more reliable data on the barriers and facilitators of achieving a healthy diet within visually impaired populations. Results indicated that 73% of those who consumed ready meals would be happy to pay over £4 for them, however due to the selection bias within the study; it is unknown whether these findings would be different in a larger, more diverse cohort of subjects.

5.5 Summary

Ready meals are popular with subjects who suffer with sight impairment, who benefit from the ease and convenience that these meals offer. However, the general perception of these meals is that they are 'unhealthy' and of lower standard to home cooked meals which contain fresh produce and no addition of manufactured ingredients such as additives and preservatives. AMD patients are keen to improve their diet and ready meals which are nutritionally tailored to support the needs of their condition by containing vegetables which provide high levels of L/Z may be a popular tool to doing so. To increase overall interest in these ready meals it is required that meals are produced with an improved nutritional profile compared with conventional supermarket sold ready meals, which can be low in calories and high in other macro-nutrients.

What this study found

- A large proportion of AMD patients currently eat ready meals due to the convenience they offer
- These benefits were further heightened in those who lived alone and struggled with the concept of cooking with sight impairment
- Those who did not consume ready meals are concerned with the 'unhealthy' connotation that surrounds them, however many of these subjects also had a spouse or family member who cooks for them
- Despite this, nearly all AMD subjects of this cohort would consume ready meals that contained kale and/or that were nutritionally tailored to support the needs of their condition

Ready meals to create based on these findings

- Recipes must have an improved nutritional profile which differs from conventional supermarket sold ready meals
- The majority of patients are happy to pay over £4 for a ready meal and usually source them from the local supermarket
- Recipes must include ingredients which contain adequate sources of L and Z such as kale, an ingredient subjects are happy to have included in their meals
- Consideration should be given to recipes that contain no additives or preservatives, are not low in calories and are not classified as high in fat, salt, or sugar
- Fish, meat and vegetarian dishes were all popular with subjects – some subjects did not like to eat red meat too often
- Popular examples of such meals included soups, casseroles, fish pie, cottage pie, lasagne, curry and creamy pasta dishes
- A small portion of subjects had certain concerns with their meals due to impaired vision such as meat or fish containing bones, or meals that had finely chopped ingredients which are very mixed together.

In this chapter we found that the usage of ready meals in AMD patients is generally high due to the convenience and simplicity that these meals offer. Patients expressed a keen interest in the production of ready meals which are nutritionally tailored to support the needs of their condition. Chapter six will describe the design of such meals, and their evaluation and impact in a sample of AMD patients.

Chapter 6: Production and evaluation of ready meals for AMD patients

6.1 Background and rationale

Collaboration with the food production company 'Lodge Farm Kitchen' (http://www.lodgefarmkitchen.com/) was formed to enable the production of ready meals which were nutritionally tailored to support the needs of those suffering with AMD. Lodge Farm Kitchen were chosen as the ready meal manufacturer as they are a small family run business who specialise in creating fresh, hand-prepared high quality food. Located in a valley in rural Herefordshire, UK, the company grow many of their own vegetables and herbs, or source them from local farmers to ensure nutritious fresh produce without the addition of additives and preservatives. According to their website they claim 'our mission at Lodge Farm Kitchen is to provide customers with convenient, tasty, nutritious frozen meals made from fresh ingredients sourced locally whenever we can – all hand prepared with pride, passion & integrity'.

Once cooked by the team at Lodge Farm Kitchen the meals are blast frozen on the same day to retain their nutrition and flavour. Flash or blast freezing refers to the process in various industries whereby objects are frozen in a few hours by subjecting them to cryogenic temperatures [344]. In the food industry this is used to prevent perishing of food items [345]. Lodge Farm Kitchen are identified by their home cooked meals which contain only ingredients used in conventional cooking and no addition of additives, preservatives or other chemical enhancers used for taste and preservation. The meals are convenient because they have a twelve-month shelf life from the date cooked and can be heated and served from frozen in a matter of minutes. Lodge Farm Kitchen supply wholesale ready meals to shops, delicatessen and supermarkets, or personal ready meals delivered to individuals. The current product range includes meat dishes such as casseroles, curries and pastas, fish dishes such as fish pie and fish soup and vegetarian meals such as soups, lasagnes and curries.

6.2 Production of ready meals

Based on the results of the survey, the ready meals which were created for AMD patients included various soup options such as a curried potato and kale soup, pea and ham soup, and a leek, potato and kale soup. It is likely that soup was a predominant choice of meal for AMD subjects as it easy to eat, easily digested and does not contain a mixture of textures and food items that may be unrecognisable to those with impaired vision. Other meals which were created to support the nutritional needs of AMD patients based on the findings from the ready meal survey included a

fish pie with spinach, cottage pie with kale, Normandy pork casserole, chicken ham and leek casserole and a creamy kale and smoky bacon pasta. All meals contained adequate amounts of additional food items high in L and Z such as eggs, kale, spinach and other vegetables. Kale was added to the meals at the very end of their preparation to ensure only minimal cooking occurred and to retain as much of its nutrient value as possible. Within ready meal dishes this method of cooking kale would have been similar to blanching, as found in the previous analytical studies (chapters 4 and 5) retention of L was maximum with shorter cooking times, lower temperatures and minimal amounts of water. A small amount of fat in the form of butter or olive oil was also added to the meal recipes to improve the bioavailability of L.

The meals were created by the Lodge Farm Kitchen team after several meetings where results of the qualitative (focus group and questionnaire chapters six and seven) and quantitative (kale L analyses chapters four and five) were discussed. It was decided that at least 5 mg of lutein per meal would be sufficient to support the recommended daily amount of 10 mg per day to improve MP levels and visual function in AMD patients [98, 99, 130-133], and delay the progression of AMD [10] as found in one large scale epidemiological study. Results from the laboratory study investigating L concentrations in kale upon various pre-and post-harvest factors (chapters four and five) assisted with determining the amount of kale needed to ensure adequate concentrations of L per meal, as this was the first study to investigate the L concentrations from raw kale grown and sold in the UK (table 3.7).

Based on the HPLC laboratory findings 100g of raw minimally processed retailed kale contained on average 12 mg of L, whereas freshly harvested kale contained on average 21 mg of L. Therefore it was proposed that one third of these portions (33.3 g of kale) would provide at least 5 mg of L per meal. For spinach, average L concentrations per 100 g of fresh weight were extracted from previous data. A study which investigated the L content of vegetables and vegetable products, fruits as well as eggs available on the Polish market using HPLC analysis, found that raw spinach contained a mean L content of 8.95 mg/100 g [346]. Moreover, a recent study which analysed the L content of a batch of fresh baby leaf spinach (Spinacia oleracea) purchased from a local retailer in Migros, Switzerland found that the whole leaves contained an average of 11.08 mg/100 g [347]. For the purpose of the ready meal design, we attained from this data that on average 100 g of fresh raw spinach would contain around 10 mg of L. This would mean that 20 g of raw spinach would contain an average L concentration of 2 mg.

Eggs are also considered a good source of L and Z, and may have enhanced bioavailability of these carotenoids in this form because of the co-ingestion with fat in which they contain [96]. The chicken egg yolk, a matrix composed of digestible lipids, i.e., cholesterol, triglycerides, and phospholipids, contains L and Z along with other fat-soluble micronutrients such as vitamin A, vitamin D, and vitamin E. A randomised controlled trial found that in older adults over 60 years of age, five weeks of consuming one egg per day significantly increased serum L (26% increase) and Z (38% increase) concentrations without elevating serum lipids and lipoprotein cholesterol concentrations [348]. Two other reports examined the same human subjects (mean age 62 y, range: 46–78 y) and showed a 28–50% increase in plasma L and a 114–142% increase in plasma Z concentrations [349] following a diet supplemented with 1.3 eggs/day for 4.5 weeks. However, this increase was associated with an 8–11% increase in plasma LDL cholesterol (LDL-C) concentrations [350].

In terms of MP levels, a study assigned twenty-four females, between 24 and 59 years, one of two egg treatments for 12 weeks. Individuals consumed six eggs per week, containing either 331 mg (EGG 1) or 964 mg (EGG 2) of L and Z per yolk. MPOD increased in both the EGG 1 ($P = 0.001$) and EGG 2 ($P = 0.049$) groups [102]. However, concentrations of L and Z in egg yolk are reported to be low in comparison to dark green leafy vegetables. For instance, a study which investigated the L content of vegetables and vegetable products, fruits as well as eggs available on the Polish market using HPLC analysis found that the L content of egg yolk ranged from 0.71 to 2.82 mg/100 g and varied depending on the method of chicken farming. The mean L content of egg yolk from battery hens was 1.07 mg/100 g whereas the mean L content for hens bred on a rural farm were almost 50% higher at 2.08 mg/100 g [346]. The results of this study are consistent with those of other authors (Leth et al., 2000 [351]; Ollilainen et al., 1989 [352]; O'Neill et al., 2001 [353]). A study carried out in Denmark showed that egg yolks from the "organic farms" had more than a twofold higher amount of L compared to yolks from battery hens and threefold higher in relation to yolks from free – living hens 1320, 527 and 384 g/100 g, respectively) [351]. Although the concentrations of L and Z in one egg yolk may be modest relative to other sources, such as kale and spinach, their bioavailability to the serum and retina appears to be high [102]. Therefore, both eggs and spinach, sourced from local organic farms were used as additional ingredients in the ready meals to further increase L and Z concentrations and their bioavailability from these food sources.

	Amount	Shop Kale	Farm Kale	Spinach
Average Lutein	100g	12mg	21mg	10mg
	10g	1.2mg	2.1mg	1mg
	30g	3.6mg	6.3mg	3mg
	5g	0.6mg	1.05mg	0.5mg

Table 3.7: Lutein quantifications for farm and shop kale based on the HPLC study; lutein quantifications for spinach based on previous data.

8.3 Nutritional analyses of ready meals using Al la Calc

Curried Soup			Normandy Pork Casserole		
Name	per 300g serving	unit	Name	per 350g serving	unit
Energy	755	kJ	Energy	1696	kJ
Energy	381	kcal	Energy	404	kcal
Fat	5.3	g	Fat	15.6	g
of which saturates	2.9	g	of which saturates	5.5	g
Carbohydrate	35.5	g	Carbohydrate	24.1	g
of which sugars	12.2	g	of which sugars	8.5	g
Fibre	8.1	g	Fibre	4.1	g
Protein	6.1	g	Protein	40	g
Salt	1	g	Salt	1.8	g
Sodium	384.34	mg	Sodium	700.96	mg
Zinc	1.2	mg	Zinc	6.3	mg
Selenium	3.2	µg	Selenium	55.7	µg
Beta Carotene	2324	µg	Beta Carotene	3526.4	µg
Vitamin D MCG	Trace	µg	Vitamin D MCG	Trace	µg
Vitamin D IU	Trace	IU	Vitamin D IU	Trace	IU
Vitamin E	1.2	mg	Vitamin E	1.8	mg
Vitamin B6	0.4	mg	Vitamin B6	1.1	mg
Vitamin B12	Trace	µg	Vitamin B12	1.6	µg
Folate	84.9	µg	Folate	153.9	µg
Vitamin C	53	mg	Vitamin C	64.2	mg
L/Z	3.4	mg	L/Z	6.6	mg

Kale & Smoky Bacon Pasta		
Name	per 350g serving	unit
Energy	1903	kJ
Energy	451	kcal
Fat	16	g
of which saturates	7.7	g
Carbohydrate	56.9	g
of which sugars	9.7	g
Fibre	4.1	g
Protein	21.9	g
Salt	1.3	g
Sodium	501.25	mg
Zinc	2.2	mg
Selenium	11.8	µg
Beta Carotene	2629	µg
Vitamin D MCG	0.4	µg
Vitamin D IU	16.5	IU
Vitamin E	1.4	mg
Vitamin B6	0.3	mg
Vitamin B12	1.6	µg
Folate	101.4	µg
Vitamin C	42.9	mg
L/Z	4.3	mg

Pea and Ham Soup		
Name	per 300g serving	unit
Energy	874	kJ
Energy	308	kcal
Fat	5.6	g
of which saturates	2.6	g
Carbohydrate	28.7	g
of which sugars	8.7	g
Fibre	9	g
Protein	13.9	g
Salt	1.6	g
Sodium	639.66	mg
Zinc	2.3	mg
Selenium	5.5	µg
Beta Carotene	2575.3	µg
Vitamin D MCG	0	µg
Vitamin D IU	0	IU
Vitamin E	0.9	mg
Vitamin B6	0.5	mg
Vitamin B12	0.3	µg
Folate	149	µg
Vitamin C	82.9	mg
L/Z	6.4	mg

Cottage Pie		
Name	per 350g serving	unit
Energy	1142	kJ
Energy	370	kcal
Fat	4.1	g
of which saturates	1.8	g
Carbohydrate	38.1	g
of which sugars	7.8	g
Fibre	6.2	g
Protein	22.5	g
Salt	1.5	g
Sodium	607.4	mg
Zinc	4.1	mg
Selenium	5.8	µg
Beta Carotene	4237.7	µg
Vitamin D MCG	0.4	µg
Vitamin D IU	14.9	IU
Vitamin E	2.2	mg
Vitamin B6	0.8	mg
Vitamin B12	1.5	µg
Folate	110	µg
Vitamin C	98.4	mg
L/Z	3.1	mg

Chicken Ham & Leek Casserole		
Name	per 434g serving	unit
Energy	2339	kJ
Energy	554	kcal
Fat	15.6	g
of which saturates	5.4	g
Carbohydrate	23.1	g
of which sugars	3.8	g
Fibre	3.5	g
Protein	85.4	g
Salt	1.8	g
Sodium	1508.15	mg
Zinc	3.6	mg
Selenium	40.7	µg
Beta Carotene	3665.7	µg
Vitamin D MCG	0.5	µg
Vitamin D IU	19.2	IU
Vitamin E	1.4	mg
Vitamin B6	1.7	mg
Vitamin B12	0.5	µg
Folate	108.4	µg
Vitamin C	50	mg
L/Z	3.5	mg

Fish Pie with Spinach			Leek Potato & Kale Soup		
Name	per 350g serving	unit	Name	per 300g serving	unit
Energy	1928	kJ	Energy	1126	kJ
Energy	458	kcal	Energy	368	kcal
Fat	16.5	g	Fat	12.7	g
of which saturates	5.8	g	of which saturates	5.5	g
Carbohydrate	36.1	g	Carbohydrate	26.6	g
of which sugars	4.5	g	of which sugars	9.4	g
Fibre	3.6	g	Fibre	3.2	g
Protein	42.1	g	Protein	15.8	g
Salt	1.5	g	Salt	1.7	g
Sodium	576.91	mg	Sodium	685.16	mg
Zinc	2	mg	Zinc	1.6	mg
Selenium	55.9	µg	Selenium	16.1	µg
Beta Carotene	1725.8	µg	Beta Carotene	2442.2	µg
Vitamin D MCG	3.7	µg	Vitamin D MCG	1.9	µg
Vitamin D IU	147.4	IU	Vitamin D IU	76.8	IU
Vitamin E	3.3	mg	Vitamin E	2	mg
Vitamin B6	0.7	mg	Vitamin B6	0.5	mg
Vitamin B12	5.2	µg	Vitamin B12	2.8	µg
Folate	127	µg	Folate	130.5	µg
Vitamin C	32.5	mg	Vitamin C	52.8	mg
L/Z	3.3	mg	L/Z	3.8	mg

Table 3.8: Nutritional analysis of each ready meal recipe using Al La Calc (Red Hot Rails LLP, Doncaster, UK) dietary analysis software. Only key macro-nutrients and nutrients important for AMD are included. Full Al La Calc results of these nutritional analyses can be found in the appendices.

The ready meal recipes were nutritionally analysed using A La Calc, for numerous nutrients, calorie values and other constituents using the USDA (United States Department of Agriculture) SR25 food database (http://ndb.nal.usda.gov/). This provided further data on the L/Z quantifications per meal and on the quantities of total calories, total fat, of which saturates, carbohydrates, fibre, protein, salt, and information on important vitamins and minerals for the eyes such as zinc, beta-carotene, vitamin C, vitamin E, vitamin D, vitamin B6, vitamin B12, and folate. Al La Calc recognised 100 g of spinach to contain 12 mg of L/Z, slightly higher than some previous studies have reported. For kale, A La Calc recognised 100 g of fresh raw kale to contain 8.9 mg of L/Z, slightly lower than our HPLC lutein study determined. This information was taken in to consideration when interpreting the nutritional analysis data on L/Z from Al La Calc; for instance meals which contained kale were recognised as containing slightly more L/Z than the software suggested. For these

purposes, information on L levels in kale as determined by the previous HPLC study (chapter four) was used as a reference for estimating the L levels in the meals, as this was the same kale sourced in the ready meals.

Ready meal	Kale (g)	Spinach (g)	Egg (g)	L shop (mg)	L farm (mg)	L spinach (mg)	L/egg (mg)	Total L (mg)
CS	35	0	0	4.2	7.35	0	0	4.2-7.35
NPC	0	50	0	0	0	5	0	5
KSBP	30	15	0	3.6	6.3	1.5	0	5.1-7.8
PHS	20	15	0	2.4	4.2	1.5	0	3.9-5.7
CP	35	0	0	4.2	7.35	0	0	4.2-7.35
CHLC	35	0	0	4.2	7.35	0	0	4.2-7.35
FP	10	20	60	1.2	2.1	3	2	6.2-7.1
LPKS	30	0	60	3.6	6.3	0	2	5.6-8.3

Table 3.9: Lutein amounts for each ready meal dependent on their ingredients and kale source. (CS- Curried Soup, NPC- Normandy Pork Casserole, KSBP- Kale and Smoky Bacon Pasta, PHS- Pea and Ham Soup, CP- Cottage Pie, CHLC- Chicken, Ham and Leek Casserole, FP- Fish Pie, LPKS- Leek, Potato and Kale Soup).

Table 3.9 displays the content of kale, spinach and eggs in each ready meal and the subsequent L concentrations from each food source. Total L concentrations of each meal varied depending on whether or not the kale was sourced directly from the farm or within its shelf life constituency purchased from a supermarket. All ready meal recipes contained kale, spinach or eggs, or a combination of these food sources. The amount of kale present in the meals varied and was dependent on the addition of spinach and eggs. For instance, lower amounts of kale were found in the meals which contained additional measures of these alternate food items. The resultant L amounts in each meal were lower than the anticipated 5 mg per meal if the kale was sourced from a local supermarket (3.6-6.2 mg per meal) therefore it was decided that all meals would contain kale that was sourced within 24 hours of harvest from a local farm, as this would increase the final L amounts to at least 7 mg per meal.

Nutritional profiles of the ready meals were compared with the UK government DRV's for males and females of those aged over 50 years [354], displayed in the following table.

	Unit	DRV F >50	DRV M >50
Energy	Kcal	1840-2079	2294-2581
Fat	g	70	95
of which saturates	g	20	30
Carbohydrate	g	230	300
of which sugars	g	90	120
Fibre	g	24	24
Protein	g	53	53
Salt	g	6	6
Zinc	mg	7	9.5
Selenium	µg	60	75
Beta Carotene	µg	-	-
Vitamin D	IU	400	400
Vitamin E	mg	10	10
Vitamin B6	mg	1.2	1.4
Vitamin B12	µg	1.5	1.5
Folate	µg	200	200
Vitamin C	mg	40	40

Table 4.1: UK female and male DRV's for important macro-nutrients and vitamins and minerals associated with AMD research. There are currently no DRV's for beta-carotene.

Overall ready meals were categorised as being low in saturated fat and salt, and low in sugar according to current UK guidance, front-of-pack traffic light nutritional labelling [355]. This is unlike previous work, where it has found that ready-meals in the UK tended to be high in saturated fat and salt [340]. All ready meals aside from the kale and smoky bacon pasta (curried soup, Normandy pork casserole, pea and ham soup cottage pie, chicken, ham and leek casserole, fish pie and leek, potato and kale soup) contained below 6 g of saturated fat per whole portion size of 300-350 g. This was substantially below the maximum recommended daily allowance of 20 g per day. The kale and smoky bacon pasta contained approximately 6.7 g of saturated fat per whole meal size of 350 g. The slightly higher levels of saturated fat in this recipe may be attributed to the creamy nature of the dish, something subjects expressed their liking towards in the ready meal survey (chapter seven). Evidence indicates that saturated fat intake should be no more than 7% of total daily energy intake [356], this is based primarily on the prediction of a progressive reduction in cardiovascular disease risk associated with greater reductions in LDL cholesterol [357]. As 1 g of saturated fat equates to 9 kcal, 7% of the average recommended 2000 kcal would be 15.5 g of saturated fat per day for females and 19.4 g of saturated fat per day for

males requiring 2500 kcal per day. Given these findings, it seems reasonable to suggest that all meals contained appropriate low amounts of saturated fat.

All eight meals included fewer than 2 g of salt per portion, which represents only 33% of the recommended daily allowance of salt. There is an overwhelming body of evidence to suggest that dietary salt is the major cause of hypertension and that a reduction in salt intake lowers blood pressure, thereby, reducing blood pressure-related diseases, [358] [359-361]. For instance, several lines of evidence including population, and prospective cohort studies, as well as outcome trials, demonstrate that a reduction in salt intake is related to a lower risk of cardiovascular disease [362-368]. It was therefore of increased importance that all meals had a low salt profile. In terms of calorie amounts, each meal contained between 300 and 600 kcal per whole portion size, which are adequate values for one meal if consumers abide by the general notion of three meals per day, plus snacks. All soups (curried soup, pea and ham soup, leek potato and kale soup) were lower than other dishes in calories, containing around 300 kcal per 300 g, as to be expected from a liquidised food source such as soup which is typically accompanied by a carbohydrate food item as a side dish. The chicken, ham and leek casserole was the highest in calories, containing 554 kcal per 350 g, which may be attributed to the two meats and small amount of chicken fat in which it contained. Despite this, it encompassed only 5.5 g of saturated fat per whole meal size of 350 g. This was substantially below the recommended maximum limit of 20 g per day and therefore was considered balanced in its nutritional profile.

Zinc levels were particularly high in the Normandy pork casserole (6.3 mg) and the cottage pie (4.1 mg), providing over half of the DRV for both males and females. In the previous diet analysis study within a sample of AMD patients (chapter two) male and female zinc consumption was similar to the DRV. Zinc has been investigated with regard to its potential preventative role in AMD. The AREDS group found a suggestive reduction in the risk of progression of AMD in participants supplementing with 25 mg zinc daily [10]. A small number of cohort studies assessing dietary intakes of zinc in elderly subjects have also found an inverse relationship between high zinc intake and the incidence of early AMD [150, 175]. All meals were additionally high in vitamin C due to their increased quantities of vegetables. As well as typically being high in the carotenoids L and Z, research has shown that dark green vegetables such as collard greens (kale) and spinach also contain high concentrations of vitamin C [257, 369]. Seven out of eight meals contained concentrations of vitamin C which were above

the DRV of 40 mg per day. This was a positive attribute to the meals given the protective antioxidant properties of vitamin C in the human retina [370].

It is important to note that micronutrients do not feature in the FSA traffic light labelling of food products and the DRV standards used are based on average intakes over time rather than on individual meals. Therefore it is not necessarily the case that one main course should meet these standards. However, in the absence of international criteria for the nutritional content of individual meals, these standards are the best currently available and have been used previously for assessment of individual products [371], [372].

Overall, it was found that the ready-meals created for AMD patients were a healthier alternative to conventional supermarket meals which are available within the ready-meal sector. In particular, it was found that meals were rated as low in fat, saturated fat and salt, and were therefore much more likely to achieve 'low' ratings of all four front-of-pack nutrients. As well as this balanced supply of key macro-nutrients, most importantly the ready meals contained substantial amounts of L and Z, as well as antioxidant micro-nutrients such as zinc and vitamin C, which may be of benefit in the primary prevention of the onset or progression of AMD.

Kale cup weights

According to the USDA National Nutrient Database for Standard Reference Release 26, the weight for one cup of raw, chopped kale is 67 grams - among the highest for raw leafy vegetables. For spinach, the USDA reports that one cup of raw baby leaf spinach is 30 grams [373]. Therefore, if we assume that there are 10 – 20 mg of L in 100g raw kale (chapter four: dependant on source) and that a cup of kale weighs 67 g, then one cup of raw kale should be enough to provide around 10 mg of L per day. Moreover, if we assume that one cup of spinach weighs 30 g and that there is 10 mg of L in 100 g of raw spinach then this should equate to 3 mg of L. However, during creation of the ready meal recipes contradictions in these findings were apparent. To ensure at least 30 g of kale per meal, kale and spinach were initially weighed in a standardised measuring cup before being added to a recipe. The findings revealed that one cup of raw, chopped kale with hard stems removed, weighed approximately 20 g. Interestingly, these findings correspond with a recent study to suggest that the USDA recommendations are in fact not a true representation of kale cup sizes [374]. Based on the USDA data, one current dietary suggestion for AMD patients is to consume at least one cup of kale per day to provide enough L to support the prevention of onset or progression of AMD. However if 20 g of kale is a true

representation of cup size, then our HPLC data indicates that this would only provide 3 mg of L, which may be insufficient for AMD patients. If patients were to try and increase their dietary L intakes without the use of supplements, they would need to consume just over three cups of shop purchased kale per day to ensure a sufficient supply of L. Patient nutritional information resources such as those given out by the MS may therefore need to be updated in connection with these findings. Further research in regard to the quantification of L within other raw leafy green vegetables sourced from the UK and verification of their cup weights may also be needed to warrant realistic dietary recommendations for AMD patients.

6.4 Delivery and evaluation of ready meals

Once hand prepared by the team at Lodge Farm Kitchen, the ready meals were blast frozen and then delivered to the corresponding research institute on the same day. Once received by the researcher they were instantly transported for a taste testing session with AMD subjects at a local MS peer support group. The MS peer support group, located in Barnt Green, Birmingham, UK, included some of the same MS members used in the focus group study. Forty-Five MS members were present at the group during the taste testing session. All participants suffered with some form of AMD. A brief introduction was given to the group before the ready meals were served, to explain the research behind the ready meals, their nutritional quality, the company Lodge Farm Kitchen and the aims and objectives of the study. Participants were made aware that they would be required to give basic feedback on the meals during the session and were therefore encouraged to try as many of the dishes as possible to support the research.

Three to four meals of each recipe were cooked following the relevant instructions and smaller samples of each dish were served to the group. Meals were cooked from frozen using an oven and microwave. A list of ingredients and cooking instructions for the meals was also given to each table of participants. Once participants had finished tasting the meal samples, an organoleptic test sheet was given to each participant for verbal completion with the help of a scribe. The test required participants to give a mark out of five for appearance, smell, texture, and taste, as displayed in the below figure. Only subjects who felt comfortable with their visual acuity were required to score for appearance. At the end of the taste testing session a brief talk was given to the participants. During the talk subject's overall opinion of the meals was discussed, where subjects were encouraged to speak out if they had something they would like

to say about the meals. Samples of each ready meal in its original packaging and full size were also given out to subjects to gain feedback on portion sizes and packaging. Participants were asked whether or not each dish would be an adequate portion for one meal and whether or not the packaging, cooking instructions and ingredient lists were easy to comprehend. Points raised by the group were transcribed.

Lodge Farm Kitchen	Organoleptic Test 1-5 (5 very good, 4 good, 3 average, 2 poor, 1 very poor)				
Product	Visual	Smell	Texture	Taste	Comment
Chicken, ham & leek casserole					
Curried potato apple & kale					
Fish pie with spinach					
Kale & smokey bacon pasta					
Leek potato & kale soup					
Normandy pork casserole					
Pea and ham soup					
Cottage Pie					

Figure 3.6: Organoleptic test sheet used to gain patient feedback on the ready meals.

Taste testing results

		Chicken Casserole	Curried Kale Soup	Fish Pie	Kale Pasta	Leek & Kale Soup	Pork Casserole	Pea Soup
Number of Tasters		*9*	*7*	*8*	*8*	*7*	*7*	*7*
Total Score	Visual	29	27	31	28	30	29	21
	Smell	40	32	33	27	32	30	23
	Texture	43	31	36	25	32	27	27
	Taste	43	32	36	27	32	27	30
		Chicken Casserole	**Curried Kale Soup**	**Fish Pie**	**Kale Pasta**	**Leek & Kale Soup**	**Pork Casserole**	**Pea Soup**
Average Score	Visual	3	4	4	4	4	4	3
	Smell	4	5	4	3	5	4	3
	Texture	5	4	5	3	5	4	4
	Taste	5	5	5	3	5	4	4

Table 4.2: Results of the taste testing session with ready meals including total and mean organoleptic scores per meal.

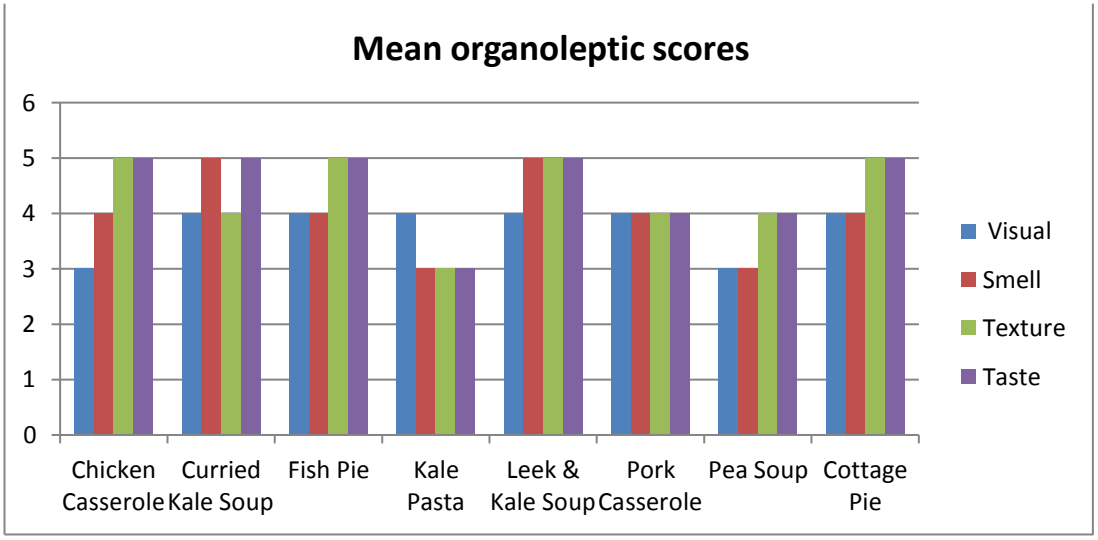

Figure 3.7: Mean organoleptic test scores for smell, taste and texture of each ready meal.

Table 4.2 displays the results of the organoleptic test used to test the ready meals created for AMD patients. The test was used in a group of forty-five AMD patients from a local MS peer support group. The number of tasters for each meal is displayed at the top of the table in red. Total and mean scores for appearance, smell, texture

and taste are displayed for each meal. Figure 3.7 displays the mean results of the organoleptic test for visual appearance, smell, texture and taste. Results showed that the chicken casserole, curried soup, fish pie, leek soup and cottage pie all had the highest mean score of 5 for taste, which represented the patient opinion of 'very good'. The pork casserole and pea soup had a mean score of 4 for taste which represented 'good' and finally the kale and smoky bacon pasta had a mean score of 3 which represented an 'average' mark. The kale and smoky bacon pasta also had the lowest scores for smell and texture out of all ready meals available. Many patients made comments during the test to suggest that the pasta was slightly dry in taste and texture which may justify these findings. The kale pasta was the only ready meal which was cooked in the microwave as opposed to the oven, which may have contributed to the dry texture. All meals may be oven or microwaved cooked from frozen, however as we were preparing samples on a large scale both cooking techniques were utilised to save time and space.

Aside from the kale and smoky bacon pasta, meals scored a 4 or above for texture and taste which signified patient's overall satisfaction with the meals. Comments from patients about the ready meals in general were excellent, with many subjects giving highly positive feedback in regard to taste and freshness. For smell, most ready meals had an average score of 4 or above, aside from the kale pasta and the pea and ham soup. Both the pea and ham soup and kale and smoky bacon pasta scored an average of 3 for smell. Some patients stated that the slightly unpleasing smell did not affect the taste of the meals.

For appearance, meals scored a 4 for 'good' aside from the kale and smoky bacon pasta and the pea and ham soup. Only subjects who felt their vision was good enough to judge a meal by its appearance gave a score for visual attributions. In regard to the kale and smoky bacon pasta, some subjects gave comments that it appeared dry and crispy in texture. Subjects also mentioned that the pea and ham soup appeared very thick and gloopy. The dry appearance of the pasta again may be attributable to the cooking method used.

Feedback for group questions after taste testing session:

Upon completion of the taste testing session, participants were asked to partake in a group discussion in order to gain some oral feedback on the ready meals. Questions relating to taste and enjoyment were propositioned to the group to gather a more

rounded view. Feedback on portion sizes and whether or not the packaging, cooking instructions and ingredient lists were easy to read and understand was also invited from the group. Comments from participants about the ready meals in general were excellent, with many subjects giving favourable responses. Subjects were in agreement that the meals did not taste like standard supermarket purchased ready meals and were more comparable in taste and flavour to home cooked meals. Participants were enthused by the notion that meals contained no additives or preservatives, were cooked using locally sourced fresh ingredients and methods which ensured optimisation of their nutritional value.

Portion sizes of the meals were approximately 300g for the soup dishes and 350g for all other meals. Whole meal samples in their original packaging were passed around the group so subjects could get a better idea of portion size. All subjects were in agreement that the portion sizes of the dishes were substantial for one whole meal. Cooking instructions and ingredient lists for each meal were handed out with the corresponding dish; a large clear bold text font in black was used on a yellow background which enabled subjects to interpret the details with ease. This colour contrast between text and background is used within all MS resources as a typical black to white contrast may cause glare in those with visual impairments.

Limitations

One limitation to this study may be the possibility of response bias resulting from the experimental design. A brief introduction was given to the group before the ready meal analysis, which explained the research behind the ready meals, their nutritional quality, the company Lodge Farm Kitchen and the aims and objectives of the study. Such circumstances may have led to a nonrandom deviation of the answers from their true value, as subjects may have believed to have understood the experiment and its expected findings, so adapted their responses to suit. This may have prompted them into giving answers skewed towards the researchers own opinions, prejudices and values [375, 376]. This type of bias should have be factored into the experiment and the amount of information given to the subjects restricted, which would have prevented them from understanding the full extent of the research.

There are several limitations to using food composition databases (such as Al la Calc) to accurately predict nutrient values in food sources. One of these may be the variability in the composition of foods, as being biological materials foods exhibit

natural variations in the amounts of nutrients contained [377]. A database is unlikely to predict within narrow limits the composition of a particular sample of food, because the limits will vary according to the source of food item and to the nutrient [378]. Al La Calc quantified the levels of L and Z together, which made it difficult to use the HPLC determination of L levels within kale (chapter 4) as a comparison for the ready meal nutrient composition tables produced by Al La Calc. As the kale used in the ready meals was sourced from the same farm investigated in the analytical study, estimates for the L concentrations of these meals were derived from the calculations of this study and not Al la Calc.

However, proposed L concentrations of these recipes should still be taken with caution as this natural variability in the composition of foods is increased by different methods of plant storage, processing and treatment. Processed foods such as ready meals, despite being subject to quality control during production, may vary, in part because of variations in the composition of ingredients but also because of changes in formulation and production [276]. Kale was added to meals at the end of their preparation to ensure only minimal cooking occurred and to retain as much of the nutrient value as possible, as this was similar to the blanching method used the previous analytical study where the retention of L was highest with shorter cooking times, lower temperatures and minimal amounts of water. In this previous study however, kale was analysed for L concentrations individually and not within the ready meal source. Furthermore, the extent to which leaching occurred was not analysed within the previous report. Determining the L content of the water after the cooking process would have given further reliable information about the proposed L content of these meals.

6.5 Summary

A taste testing session was conducted with a group of forty-five AMD patients from a local Macular Society peer support group. All ready meals designed with the help of Lodge Farm Kitchen were included in the evaluation session and subjects were required to give scores out of five for appearance, taste, texture and smell using an organoleptic test method. Oral feedback was gained from the subjects during a group session after the testing to distinguish overall opinions of the meals and the suitability of portion sizes. The ease at which subjects could comprehend the corresponding ingredient lists and cooking instructions for meals was also discussed. Out of the total number of eight ready meals, seven scored four (good) or above for taste, with the

exception being the kale and smoky bacon pasta which scored a three (average). All meals also scored a three or above for other factors such as appearance, smell and texture and overall comments from study group members in regard to the meals were excellent. To improve the taste and texture for the lowest scoring meal of the kale and smoky bacon pasta it may be necessary to recommend oven cooking as opposed to microwave cooking in order to prevent meal dryness. Feedback from the group discussion informed us that portion sizes were suitable for subjects and ingredient lists and cooking instructions were easily read and understood. Subjects were further enthused by the facts that meals contained no additives or preservatives, were cooked using locally sourced fresh ingredients and methods which ensured optimisation of their nutritional value. This evaluation study gives insight in to the use of ready meals as a novel intervention to improve diet in those suffering with AMD. The highly positive feedback gained from subjects during the session will be valuable when exploring how to further develop these prototype meals and the next steps in manufacturing them on a larger scale.

Chapter 7: Discussion

Current knowledge suggests that nutrition through dietary modification or supplement use may prevent the onset or progression of AMD [8, 10]. The investigations described over the course of the previous chapters aimed to contribute new information to the literature on nutrition and AMD, with the use of various qualitative and quantitative studies which focus on optimising dietary modification for this condition. There are various limitations to the studies constructed within this thesis which need to be considered when interpreting the overall quality, validity and generalisability of results reported. However, the problems and limitations encountered provide valuable feedback to inform further development of research methods in these populations and larger scale studies of such interventions.

The peer-reviewed literature in chapter one provided a detailed review on the pre-and post-harvest effects of the carotenoid concentrations in leafy green vegetables. These findings are important because they demonstrate that there is a paucity of evidence that has investigated the pre and post-harvest processing and storage effects of kale grown and retailed in the UK, thus setting a precedent for the study in chapter three. Currently AMD patients are advised to include more dark green vegetables such as kale in to the diet to increase their intake of L and Z, which are implicated in the maintenance of retinal health and possible prevention of the onset or progression AMD [379, 380]. Therefore, information regarding their stability during domestic cooking and storage is crucial.

Notable and statistically significant findings were obtained in chapter three; L levels in minimally processed kale prepared for retail were significantly lower than that of intact whole kale sourced freshly harvested from the farm ($p<0.001$). Domestic cooking and storage were also found to have significant negative effects on the content of L in these kale sources. Reducing processing/storage time and using cooking methods which require lower temperatures and minimal amounts of water such as steaming, blanching and liquidising, improved L retention significantly. These outcomes have been reported in studies which have investigated the cooking and storage stability of other predominant carotenoid containing foods, thus vegetables such as kale should be consumed soon after harvest, or postharvest handling conditions must be controlled such that nutrient degradation does not occur [199, 277]. Further research is required to support the chemical and biochemical alterations that occur in post-harvest minimally processed foods sold in supermarkets and on the improved

maintenance of their nutritional quality, particularly for food sources that have not been rigorously investigated such as UK kale.

The UK charity the MS advocates the use of vegetables high in L and Z within their patients resources. However the dietary analysis data in chapter two indicated that patients who seek their services are under consuming nutrients regarded as important for their condition, such as L and Z [211]. The qualitative research in chapter four was designed to follow up this work and gain and understanding of the dietary behaviour of AMD patients and the possible barriers to dietary change. A focus group method was selected as this approach fosters open interaction and discussion among group members, which generated rich data related to the individual needs of older adults living with visual impairment. Data revealed that patients recognised the importance of following a diet which contains important nutrients to help slow the progression of AMD, yet altering dietary habits is proven difficult when there are underlying individual barriers to change. Chapter five expanded on this evidence by establishing, in more detail, the food choices and preferences of AMD patients by use of an open ended survey within a larger cohort. It was concluded that ready meals which are nutritionally tailored to support the needs of these individuals, may be a promising intervention for improving diet in these individuals.

The external validity of the results presented in the observational studies within this report may be questioned due to the presence of small sample sizes. One criticism of qualitative data analysis is that because it typically involves examination of data extracted from small, non-random samples, findings are usually not generalisable beyond the local research participants [375, 376]. However, what is a limitation for one purpose is a strength for another purpose. Specifically, the examination of relatively small samples allows qualitative researchers to collect maximally rich data via the methods configured within these reports (e.g. focus groups, observations, nonverbal communication and open ended surveys) [299]. This, in turn, makes it more likely that as a result of the qualitative data analysis, verstehen will be achieved.

The subject samples used in the above studies, of course, are not a truly accurate representation of all patients seeking services from the MS. More time and access to information from more patients would have provided a more rounded and reliable view. Furthermore, the overall health of subjects was not investigated within these cohorts, suggesting that results relating to issues with visual impairment may have been the cause of other contributing factors. As dietary habits are multi-factorial [381], future research taking in to consideration other cofounding variables should be

encouraged. This research solely focuses on AMD patients who are members of the UK charity the MS. Since the AMD cohorts all had a form of AMD and were motivated to contact or engage with the MS for support, it may be presumed that they were interested in preventing the disease from progressing. Thus, future research should aim to find out the opinions of those with AMD who have not sought support from professional or non-professional organisations and therefore not considered an 'informed' population.

This PhD thesis contributes new evidence to the research base within nutrition and AMD. The results were in aligning with previous studies that, in spite of advice being given to patients by the MS, they primarily eat food they enjoy and are used to [211]. Changing eating habits therefore requires novel intervention methods [210]. This project outlines the design of an effective and innovate measure for improving diet in patients with, or at risk of, AMD. The ready meals were designed based on the food likes and dislikes of AMD patients (chapter five) and on the optimum conditions for acquirement and handling of kale (chapter four). Upon the success of a taste testing session within an AMD cohort (chapter six), the MS agreed to endorse the ready meals and assist with the marketing and promotion of them through their member resources. The 'macular meals' are now available for members of the public to purchase through the Lodge Farm Kitchen website either online or via the telephone. As a result, there has been a large interest in the ready meals amongst AMD patients and other members of the public.

Results from the laboratory investigation within this report intend to enrich the current dietary advice and information given to AMD patients. Indeed, 40–80% of medical information provided by healthcare practitioners is forgotten immediately [382] and the greater the amount of information presented, the lower the proportion correctly recalled [383]. However, Ley's model on effective communication in medical practice stresses the importance of memory next to factors such as the understanding of information and satisfaction with the treatment [382]. Thus, providing further understanding on the pre and post-harvest effects of L levels in kale will enable precise suggestions for increasing retinal levels of these nutrients, which in turn, may result in improved dietary habits. Though it is important to consider that modifying a diet to include high amounts of L and Z may not have as much effect as one would hope. The reasoning behind this uncertainty stems from the knowledge that there are many uncontrollable inter-individual variations that regulate the digestion, absorption, transport and eventual retinal uptake and maintenance of L and Z [279], which haven't

been explored here. Nevertheless, results of this report will hopefully support the continued investigation of L and Z within AMD therapy.

In summary, this PhD project has enabled research to be taken from the laboratory, conducting HPLC analysis to determine the lutein concentrations of kale upon various pre-and post-harvest factors, to the patient, by producing evidence-based ready meals to improve diet in people with macular degeneration. The evidence embedded gives insight in to the use of ready meals as a novel intervention to improve diet in those suffering with AMD and provides the foundations for the manufacturing of these meals on a larger scale.

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Appendices

Appendix 1• Peer reviewed publication• Variation in carotenoid content of kale and other vegetables A review of pre- and post-harvest effects.

Link to article:
https://pubs.acs.org/doi/10.1021/acs.jafc.5b03691

Appendix 2: Focus group data analysis sheet: Matrix for assessing level of consensus in the focus group.

Focus Group Question	Member 1	Member 2	Member 3	Member 4	Member 5	Member 6
1						
2						
3						
4						
…						

The following notations were entered in the cells:

A = Indicated agreement (i.e., verbal or nonverbal)
D = Indicated dissent (i.e., verbal or nonverbal)
SE = Provided significant statement or example suggesting agreement
SD = Provided significant statement or example suggesting dissent
NR = Did not indicate agreement or dissent (i.e., nonresponse).

Appendix 3: Ready meal questionnaire for AMD patients.

1) What is your gender?

2) How old are you?

3) Do you suffer with dry AMD or wet AMD? Dry ☐ Wet ☐

4) Do you eat ready meals?

   Yes ☐ No ☐

   If answer Yes go to Q3 if answer No go to Q2

5) Can you tell me the main reasons why you do not eat ready meals?

   …… Go to Q8

6) Why do you eat ready meals?

   ……

7) How often do you eat ready meals?

   Once a month ☐ Once a fortnight ☐ 1-2 times a week ☐ 2-3 times a week ☐ 3-4 times a week ☐ Everyday ☐

8) How much would you willing to pay for a ready meal?

   < £2 ☐ £2-4 ☐ > £4 ☐

9) Can you tell me which of the following outlets you access ready meals from?

   Telephone ☐ Supermarket ☐ Website ☐ Shopping channel ☐

10) Would you consider eating a ready meal which contained kale?

    Yes ☐ No ☐

11) Would you consider eating a ready meal that was tailored to suit the nutritional needs of people with AMD?

    Yes ☐ No ☐

12) What kinds of foods do you like to eat? (In ready meals or normal meals)

    ……

13) Which of the following types of food would you like to eat?

Fish ☐ Chicken ☐ Beef ☐ Lamb ☐ Pork ☐

Vegetarian meals ☐ Food that I can eat without utensils (e.g. a pasty) ☐

One-pot meals such as soup or casserole ☐

14) If you are visually impaired can you tell me about any foods that are particularly difficult for you to eat?

……

Appendix 4: Letter inviting subjects of the Macular Societies sight impairment register to partake in the ready meal survey.

9th December 2015

Dear Macular Society member

The Macular Society is working with Aston University to develop ready meals suited towards people who are visually impaired. We are looking for Macular Society members to complete a simple questionnaire about the foods that they like to eat. To take part you must be over 55 years old and be diagnosed with AMD. You must also be able to hear and reply to questions in English over the telephone.

If you are willing to take part, please contact **Rachel Walsh**:

By phone: 0121 4511154 07572037342

By email: walshr1@aston.ac.uk

Rachel will call you back at a convenient time to complete the questionnaire over the phone.

Thank you in advance.

Appendix 5: Ingredient lists for each ready meal created.

Product	Ingredients	Weight	Gram per portion
Curried potato and kale soup	Onion	300g	36
	Potato		48
	Apple		28.5
	Kale		35
	Leek		24
	Parsnip		12
	Vegetable boullion		2
	Curry powder		0.01
	Apple cider vinegar		1.2
	Water		180
	Salt		0.02
	Pepper		0.001
	Apple juice		30
	Honey		2
	Coconut powder		10
	Crème fraiche half fat		25
Normandy pork casserole	Bramley apple	350g	35.38
	Olive oil		0.1
	Butter		0.1
	Onion		34.6
	Shoulder of pork		184.6
	Water		115.3
	Marigold vegetable boullion		3.84
	Apsall dry cider		38.46
	Leeks		26.15
	Red pepper		27.69
	New potatoes		38.46
	Spinach		**50**
	Crème fraiche half fat		8
	Cornflour		3.84
Kale and smoky bacon pasta	Pasta	350g	53.33
	Olive oil		0.05
	Smokey bacon lardons		26.66
	Cream cheese low fat		40
	Spinach		15
	Butter		5
	Milk semi skimmed		133.33
	Flour		13.33

	Kale		**30**
	Seaweed		0.333
Pea and ham soup	Peas	300g	120
	Ham		26
	Onion		20.2
	Potato		37
	Kale		20
	Spinach		15
	Water (from cooked ham)		128
	Chicken boullion		2.4
	Salt		0.02
	Pepper		0.01
	Butter		4.4
Cottage pie	Minced beef	350g	74.4
	Onion		26.66
	Tinned tomato		66.66
	Tomato puree		3.33
	Red pepper		20
	Beef boullion		3.33
	Celery		14
	Kale		**35**
	Water		33.33
	Carrot		18.66
	Cornflour		1.66
	Potato		133
	Salt		0.02
	Pepper		0.001
Chicken ham and leek casserole	Water	350g	210
	Chicken boullion		6
	Chicken breast		120
	Butter/chicken fat		4.5
	Carrots		15.5
	Kale		**35**
	Leeks		30
	Plain flour		8
	Cooked ham		45
	New potatoes		50
Fish pie with spinach	Haddock	350g	34.28
	Salmon		34.28
	Smoked haddock		34.28
	Eggs		**60**
	Flour		8.49

	Butter		14.28
	Milk		67
	Spinach		20
	Kale		10
	Potatoes		150
	Pepper		0.03
	Salt		0.3
Leek potato and kale soup	Leek	300g	65.62
	Potato		45
	Kale		30
	Water		62.5
	Vegetable boullion		4.375
	Salt		0.01
	Pepper		0.001
	Butter		6.87
	Milk		150
	Eggs		**60**
	Nutmeg		
	Arrowroot		2.08

Appendix 6: Full nutrient analyses of ready meals from Al La Calc.

Curried Soup

Name	per 100g	unit	per 300g serving	unit
Energy	177	kJ	755	kJ
Energy	42	kcal	381	kcal
Fat	1.2	g	5.3	g
of which saturates	0.7	g	2.9	g
Carbohydrate	8.3	g	35.5	g of
which sugars	2.9	g	12.2	g
Fibre	1.9	g	8.1	g
Protein	1.4	g	6.1	g
Salt	0.2	g	1	g
Sodium	89.85	mg	384.34	mg
Calories from Fat	11	kcal	47	kcal
Fatty Acids Monounsaturated	0.3	g	1.1	g
Fatty Acids Polyunsaturated	0.1	g	0.4	g
Trans Fatty Acids	trace	g	0.1	g
Starch	0.1	g	0.3	g
Water	87.6	g	374.5	g
Nitrogen	trace	g	0.2	g
Cholesterol	0	mg	0	mg
Potassium	165.8	mg	709.2	mg
Calcium	29.9	mg	127.8	mg
Magnesium	22.1	mg	94.5	mg
Phosphorus	40.7	mg	174	mg
Iron	0.7	mg	2.9	mg
Copper	0.2	mg	1.1	mg
Zinc	0.3	mg	1.2	mg
Chloride	4.5	mg	19.4	mg
Manganese	0.2	mg	0.9	mg
Selenium	0.7	µg	3.2	µg
Iodine	0.5	µg	2.3	µg
Retinol	14	µg	60	µg
Carotene	1.8	µg	7.8	µg
Alpha Carotene	4.4	µg	18.9	µg
Beta Carotene	543.3	µg	2324	µg
Vitamin D MCG	trace	µg	trace	µg
Vitamin D IU	trace	IU	trace	IU
Vitamin E	0.3	mg	1.2	mg
Thiamin	trace	mg	0.2	mg
Riboflavin	trace	mg	0.2	mg
Niacin	0.4	mg	1.6	mg
Tryptophan/60	trace	mg	0.1	mg
Vitamin B6	0.1	mg	0.4	mg
Vitamin B12	trace	µg	trace	µg
Folate	19.8	µg	84.9	µg
Pantothenic Acid	0.1	mg	0.5	mg
Biotin	0.1	µg	0.4	µg
Vitamin C	12.4	mg	53	mg
Ash	0.5	g	2.1	g

Folic Acid	0	µg	0	µg
Food Folate	18.8	µg	80.3	µg
Dietary Folate Equivalents	18.8	µg	80.3	µg
Choline	2.7	µg	11.6	µg
Vitamin A IU	961.8	IU	4113.7	IU
Vitamin A RAE	60.1	µg	257	µg
Beta Cryptoxanthin	7.5	µg	32.1	µg
Lycopene	0	µg	0	µg
Lutein Zeaxanthin	781.6	mg	3.343	mg
Vitamin K	61.3	µg	262.1	µg

Normandy Pork Casserole

Name	per 100g	unit	per 350g serving	unit
Energy	376	kJ	1696	kJ
Energy	90	kcal	404	kcal
Fat	3.5	g	15.6	g
of which saturates	1.2	g	5.5	g
Carbohydrate	5.4	g	24.1	g of
which sugars	1.9	g	8.5	g
Fibre	0.9	g	4.1	g
Protein	8.9	g	40	g
Salt	0.4	g	1.8	g
Sodium	155.64	mg	700.96	mg
Calories from Fat	31	kcal	141	kcal
Fatty Acids Monounsaturated	1.4	g	6.3	g
Fatty Acids Polyunsaturated	0.4	g	1.7	g
Trans Fatty Acids	trace	g	trace	g
Starch	2	g	8.8	g
Water	80.5	g	362.7	g
Nitrogen	trace	g	0.2	g
Cholesterol	27.5	mg	123.9	mg
Potassium	282	mg	1270.2	mg
Calcium	26	mg	117.1	mg
Magnesium	22.7	mg	102.3	mg
Phosphorus	100.4	mg	452.1	mg
Iron	1.1	mg	4.8	mg
Copper	0.1	mg	0.4	mg
Zinc	1.4	mg	6.3	mg
Chloride	11.1	mg	50	mg
Manganese	0.2	mg	0.7	mg
Selenium	12.4	µg	55.7	µg
Iodine	0.5	µg	2.1	µg
Retinol	6.3	µg	28.4	µg
Carotene	0.4	µg	1.7	µg
Alpha Carotene	1.2	µg	5.5	µg
Beta Carotene	783	µg	3526.4	µg
Vitamin D MCG	trace	µg	trace	µg
Vitamin D IU	trace	IU	trace	IU
Vitamin E	0.4	mg	1.8	mg
Thiamin	0.4	mg	1.8	mg

Riboflavin	0.2	mg	0.7	mg
Niacin	2	mg	9.2	mg
Tryptophan/60	trace	mg	0.2	mg
Vitamin B6	0.3	mg	1.1	mg
Vitamin B12	0.3	µg	1.6	µg
Folate	34.2	µg	153.9	µg
Pantothenic Acid	0.4	mg	1.9	mg
Biotin	0.2	µg	0.9	µg
Vitamin C	14.3	mg	64.2	mg
Ash	0.7	g	3.3	g
Folic Acid	0	µg	0	µg
Food Folate	31.6	µg	142.3	µg
Dietary Folate Equivalents	31.6	µg	142.3	µg
Choline	3.5	µg	15.8	µg
Vitamin A IU	1351.5	IU	6086.7	IU
Vitamin A RAE	72.9	µg	328.4	µg
Beta Cryptoxanthin	30.1	µg	135.7	µg
Lycopene	0	µg	0	µg
Lutein Zeaxanthin	1468	mg	6.6114	mg
Vitamin K	56.7	µg	255.3	µg

Kale & Smoky Bacon Pasta

Name	per 100g	unit	per 350g serving	unit
Energy	613	kJ	1903	kJ
Energy	145	kcal	451	kcal
Fat	5.2	g	16	g
of which saturates	2.5	g	7.7	g
Carbohydrate	18.3	g	56.9	g
of which sugars	3.1	g	9.7	g
Fibre	1.3	g	4.1	g
Protein	7	g	21.9	g
Salt	0.4	g	1.3	g
Sodium	161.53	mg	501.25	mg
Calories from Fat	46	kcal	144	kcal
Fatty Acids Monounsaturated	1.6	g	4.9	g
Fatty Acids Polyunsaturated	0.5	g	1.7	g
Trans Fatty Acids	trace	g	0.1	g
Starch	13.5	g	41.9	g
Water	67.1	g	208.2	g
Nitrogen	0.9	g	2.9	g
Cholesterol	18.3	mg	56.9	mg
Potassium	236.4	mg	733.7	mg
Calcium	96.3	mg	298.9	mg
Magnesium	22.7	mg	70.5	mg
Phosphorus	117.6	mg	364.8	mg
Iron	0.8	mg	2.3	mg
Copper	0.3	mg	1.1	mg
Zinc	0.7	mg	2.2	mg
Chloride	199.5	mg	619.1	mg
Manganese	0.3	mg	0.8	mg

Selenium	3.8	µg	11.8	µg
Iodine	12.1	µg	37.7	µg
Retinol	32.2	µg	100	µg
Carotene	17.3	µg	53.8	µg
Alpha Carotene	5.2	µg	16.2	µg
Beta Carotene	847.2	µg	2629	µg
Vitamin D MCG	0.1	µg	0.4	µg
Vitamin D IU	5.3	IU	16.5	IU
Vitamin E	0.5	mg	1.4	mg
Thiamin	0.1	mg	0.4	mg
Riboflavin	0.2	mg	0.5	mg
Niacin	2.3	mg	7.1	mg
Tryptophan/60	1.1	mg	3.4	mg
Vitamin B6	0.1	mg	0.3	mg
Vitamin B12	0.5	µg	1.6	µg
Folate	32.7	µg	101.4	µg
Pantothenic Acid	0.6	mg	1.9	mg
Biotin	1.8	µg	5.7	µg
Vitamin C	13.8	mg	42.9	mg
Ash	0.5	g	1.6	g
Folic Acid	0	µg	0	µg
Food Folate	25.2	µg	78.1	µg
Dietary Folate Equivalents	25.2	µg	78.1	µg
Choline	2.5	µg	7.7	µg
Vitamin A IU	1539.7	IU	4777.7	IU
Vitamin A RAE	106.2	µg	329.7	µg
Beta Cryptoxanthin	7.8	µg	24.3	µg
Lycopene	0	µg	0	µg
Lutein Zeaxanthin	1382.2	mg	4.2891	mg
Vitamin K	91.7	µg	284.5	µg

Pea and Ham Soup

Name	per 100g	unit	per 300g serving	unit
Energy	234	kJ	874	kJ
Energy	56	kcal	308	kcal
Fat	1.5	g	5.6	g
of which saturates	0.7	g	2.6	g
Carbohydrate	7.7	g	28.7	g of
which sugars	2.3	g	8.7	g
Fibre	2.4	g	9	g
Protein	3.7	g	13.9	g
Salt	0.4	g	1.6	g
Sodium	171.48	mg	639.66	mg
Calories from Fat	13	kcal	49	kcal
Fatty Acids Monounsaturated	0.4	g	1.5	g
Fatty Acids Polyunsaturated	0.2	g	0.6	g
Trans Fatty Acids	trace	g	trace	g
Starch	1.7	g	6.3	g
Water	85.4	g	318.7	g
Nitrogen	0.3	g	0.9	g

Cholesterol	6.6	mg	24.5	mg
Potassium	205.7	mg	767.3	mg
Calcium	24.6	mg	91.7	mg
Magnesium	20.9	mg	78.1	mg
Phosphorus	71.9	mg	268.1	mg
Iron	0.8	mg	2.9	mg
Copper	0.2	mg	0.6	mg
Zinc	0.6	mg	2.3	mg
Chloride	79.8	mg	297.5	mg
Manganese	0.2	mg	0.8	mg
Selenium	1.5	µg	5.5	µg
Iodine	0.5	µg	1.7	µg
Retinol	7.9	µg	29.5	µg
Carotene	trace	µg	trace	µg
Alpha Carotene	9.7	µg	36	µg
Beta Carotene	690.4	µg	2575.3	µg
Vitamin D MCG	0	µg	0	µg
Vitamin D IU	0	IU	0	IU
Vitamin E	0.2	mg	0.9	mg
Thiamin	0.2	mg	0.6	mg
Riboflavin	0.1	mg	0.3	mg
Niacin	1.5	mg	5.6	mg
Tryptophan/60	0.3	mg	1	mg
Vitamin B6	0.1	mg	0.5	mg
Vitamin B12	0.1	µg	0.3	µg
Folate	39.9	µg	149	µg
Pantothenic Acid	0.2	mg	0.6	mg
Biotin	0.2	µg	0.9	µg
Vitamin C	22.2	mg	82.9	mg
Ash	0.5	g	1.8	g
Folic Acid	0	µg	0	µg
Food Folate	37.3	µg	139.3	µg
Dietary Folate Equivalents	37.3	µg	139.3	µg
Choline	10.5	µg	39.2	µg
Vitamin A IU	1188.4	IU	4432.9	IU
Vitamin A RAE	66	µg	246.1	µg
Beta Cryptoxanthin	4.3	µg	16.2	µg
Lycopene	trace	µg	trace	µg
Lutein Zeaxanthin	1727.1	mg	6.4425	mg
Vitamin K	65.4	µg	243.9	µg

Cottage Pie

Name	per 100g	unit	per 350g serving	unit
Energy	288	kJ	1142	kJ
Energy	68	kcal	370	kcal
Fat	1	g	4.1	g
of which saturates	0.4	g	1.8	g
Carbohydrate	9.6	g	38.1	g
of which sugars	2	g	7.8	g
Fibre	1.6	g	6.2	g
Protein	5.7	g	22.5	g

Salt	0.4	g	1.5	g
Sodium	153.1	mg	607.4	mg
Calories from Fat	9	kcal	37	kcal
Fatty Acids Monounsaturated	0.4	g	1.7	g
Fatty Acids Polyunsaturated	0.1	g	0.4	g
Trans Fatty Acids	trace	g	0.2	g
Starch	6.1	g	24.1	g
Water	81.8	g	324.7	g
Nitrogen	0.8	g	3.3	g
Cholesterol	6.9	mg	27.5	mg
Potassium	341.4	mg	1354.6	mg
Calcium	24.9	mg	99	mg
Magnesium	19.4	mg	77.1	mg
Phosphorus	64.8	mg	257.3	mg
Iron	0.7	mg	2.8	mg
Copper	0.2	mg	0.8	mg
Zinc	1	mg	4.1	mg
Chloride	238.4	mg	945.6	mg
Manganese	0.1	mg	0.6	mg
Selenium	1.5	µg	5.8	µg
Iodine	2.9	µg	11.5	µg
Retinol	trace	µg	trace	µg
Carotene	69.8	µg	277.1	µg
Alpha Carotene	169.3	µg	671.7	µg
Beta Carotene	1068.2	µg	4237.7	µg
Vitamin D MCG	0.1	µg	0.4	µg
Vitamin D IU	3.8	IU	14.9	IU
Vitamin E	0.6	mg	2.2	mg
Thiamin	0.1	mg	0.5	mg
Riboflavin	0.1	mg	0.2	mg
Niacin	2.6	mg	10.3	mg
Tryptophan/60	0.9	mg	3.6	mg
Vitamin B6	0.2	mg	0.8	mg
Vitamin B12	0.4	µg	1.5	µg
Folate	27.7	µg	110	µg
Pantothenic Acid	0.3	mg	1.3	mg
Biotin	0.6	µg	2.3	µg
Vitamin C	24.8	mg	98.4	mg
Ash	0.3	g	1.2	g
Folic Acid	0	µg	0	µg
Food Folate	18.2	µg	72.2	µg
Dietary Folate Equivalents	18.2	µg	72.2	µg
Choline	1.4	µg	5.5	µg
Vitamin A IU	1879.9	IU	7458.1	IU
Vitamin A RAE	103.8	µg	411.7	µg
Beta Cryptoxanthin	31.8	µg	126.4	µg
Lycopene	292.9	µg	1162	µg
Lutein Zeaxanthin	785.1	mg	3.1147	mg
Vitamin K	65.4	µg	259.6	µg

Chicken Ham & Leek Casserole

Name	per 100g	unit	per 350g serving	unit
Energy	539	kJ	2339	kJ
Energy	128	kcal	554	kcal
Fat	3.6	g	15.6	g
of which saturates	1.2	g	5.4	g
Carbohydrate	5.3	g	23.1	g
of which sugars	0.9	g	3.8	g
Fibre	0.8	g	3.5	g
Protein	19.7	g	85.4	g
Salt	0.9	g	3.8	g
Sodium	347.5	mg	1508.15	mg
Calories from Fat	32	kcal	139	kcal
Fatty Acids Monounsaturated	1.3	g	5.8	g
Fatty Acids Polyunsaturated	0.5	g	2.3	g
Trans Fatty Acids	trace	g	0.2	g
Starch	2.9	g	12.6	g
Water	70.3	g	305	g
Nitrogen	3.1	g	13.3	g
Cholesterol	59.1	mg	256.6	mg
Potassium	346.4	mg	1503.5	mg
Calcium	26.6	mg	115.4	mg
Magnesium	28.2	mg	122.5	mg
Phosphorus	199.2	mg	864.3	mg
Iron	0.7	mg	3.1	mg
Copper	0.2	mg	0.8	mg
Zinc	0.8	mg	3.6	mg
Chloride	152.8	mg	663.3	mg
Manganese	0.1	mg	0.5	mg
Selenium	9.4	µg	40.7	µg
Iodine	4.7	µg	20.3	µg
Retinol	7	µg	30.2	µg
Carotene	trace	µg	trace	µg
Alpha Carotene	128.5	µg	557.8	µg
Beta Carotene	844.6	µg	3665.7	µg
Vitamin D MCG	0.1	µg	0.5	µg
Vitamin D IU	4.4	IU	19.2	IU
Vitamin E	0.3	mg	1.4	mg
Thiamin	0.2	mg	0.8	mg
Riboflavin	0.1	mg	0.5	mg
Niacin	11.4	mg	49.5	mg
Tryptophan/60	3.7	mg	15.9	mg
Vitamin B6	0.4	mg	1.7	mg
Vitamin B12	0.1	µg	0.5	µg
Folate	25	µg	108.4	µg
Pantothenic Acid	1	mg	4.5	mg
Biotin	1.5	µg	6.4	µg
Vitamin C	11.5	mg	50	mg
Ash	0.3	g	1.2	g
Folic Acid	0	µg	0	µg
Food Folate	16.5	µg	71.6	µg

Dietary Folate Equivalents	16.5	µg	71.6	µg
Choline	1.2	µg	5.3	µg
Vitamin A IU	1543.4	IU	6698.5	IU
Vitamin A RAE	83	µg	360.1	µg
Beta Cryptoxanthin	6.5	µg	28.4	µg
Lycopene	trace	µg	0.2	µg
Lutein Zeaxanthin	801.6	mg	3.479	mg
Vitamin K	60.6	µg	263.2	µg

Fish Pie with Spinach

Name	per 100g	unit	per 350g serving	unit
Energy	425	kJ	1928	kJ
Energy	101	kcal	458	kcal
Fat	3.6	g	16.5	g
of which saturates	1.3	g	5.8	g
Carbohydrate	8	g	36.1	g
of which sugars	1	g	4.5	g
Fibre	0.8	g	3.6	g
Protein	9.3	g	42.1	g
Salt	0.3	g	1.5	g
Sodium	127.17	mg	576.91	mg
Calories from Fat	32	kcal	147	kcal
Fatty Acids Monounsaturated	1.2	g	5.6	g
Fatty Acids Polyunsaturated	0.6	g	2.7	g
Trans Fatty Acids	trace	g	0.1	g
Starch	6.4	g	29	g
Water	77.5	g	351.8	g
Nitrogen	1.1	g	5.2	g
Cholesterol	63.6	mg	288.4	mg
Potassium	310	mg	1406.3	mg
Calcium	41.5	mg	188.1	mg
Magnesium	24	mg	108.7	mg
Phosphorus	116.2	mg	527.1	mg
Iron	0.7	mg	3	mg
Copper	0.1	mg	0.4	mg
Zinc	0.4	mg	2	mg
Chloride	121	mg	548.8	mg
Manganese	0.1	mg	0.6	mg
Selenium	12.3	µg	55.9	µg
Iodine	54.9	µg	249.1	µg
Retinol	28.8	µg	130.5	µg
Carotene	1.4	µg	6.5	µg
Alpha Carotene	1.2	µg	5.4	µg
Beta Carotene	380.4	µg	1725.8	µg
Vitamin D MCG	0.8	µg	3.7	µg
Vitamin D IU	32.5	IU	147.4	IU
Vitamin E	0.7	mg	3.3	mg
Thiamin	0.1	mg	0.7	mg
Riboflavin	0.1	mg	0.6	mg
Niacin	3.1	mg	14.1	mg

Tryptophan/60	1.4	mg	6.5	mg
Vitamin B6	0.2	mg	0.7	mg
Vitamin B12	1.1	µg	5.2	µg
Folate	28	µg	127	µg
Pantothenic Acid	0.6	mg	2.6	mg
Biotin	3.3	µg	15.2	µg
Vitamin C	7.2	mg	32.5	mg
Ash	0.2	g	1.1	g
Folic Acid	0	µg	0	µg
Food Folate	12.8	µg	58.2	µg
Dietary Folate Equivalents	12.8	µg	58.2	µg
Choline	8.1	µg	36.5	µg
Vitamin A IU	732.9	IU	3324.9	IU
Vitamin A RAE	60.8	µg	275.7	µg
Beta Cryptoxanthin	1.8	µg	8.1	µg
Lycopene	trace	µg	trace	µg
Lutein Zeaxanthin	730	mg	3.3118	mg
Vitamin K	36.9	µg	167.6	µg

Leek Potato & Kale Soup

Name	per 100g	unit	per 300g serving	unit
Energy	279	kJ	1126	kJ
Energy	66	kcal	368	kcal
Fat	3.2	g	12.7	g
of which saturates	1.4	g	5.5	g
Carbohydrate	6.6	g	26.6	g of
which sugars	2.3	g	9.4	g
Fibre	0.8	g	3.2	g
Protein	3.9	g	15.8	g
Salt	0.4	g	1.7	g
Sodium	169.93	mg	685.16	mg
Calories from Fat	28	kcal	113	kcal
Fatty Acids Monounsaturated	0.9	g	3.8	g
Fatty Acids Polyunsaturated	0.3	g	1.2	g
Trans Fatty Acids	trace	g	0.1	g
Starch	1.9	g	7.7	g
Water	84.7	g	341.6	g
Nitrogen	0.5	g	2.1	g
Cholesterol	56.7	mg	228.4	mg
Potassium	195.1	mg	786.7	mg
Calcium	68.2	mg	275.2	mg
Magnesium	16.5	mg	66.5	mg
Phosphorus	75.4	mg	303.9	mg
Iron	0.8	mg	3.2	mg
Copper	0.1	mg	0.6	mg
Zinc	0.4	mg	1.6	mg
Chloride	65.6	mg	264.4	mg
Manganese	0.2	mg	0.6	mg
Selenium	4	µg	16.1	µg
Iodine	17.2	µg	69.5	µg

Retinol	33.2	µg	133.9	µg
Carotene	2.9	µg	11.7	µg
Alpha Carotene	4	µg	16.2	µg
Beta Carotene	605.7	µg	2442.2	µg
Vitamin D MCG	0.5	µg	1.9	µg
Vitamin D IU	19	IU	76.8	IU
Vitamin E	0.5	mg	2	mg
Thiamin	0.1	mg	0.3	mg
Riboflavin	0.2	mg	0.7	mg
Niacin	1	mg	3.9	mg
Tryptophan/60	0.7	mg	3	mg
Vitamin B6	0.1	mg	0.5	mg
Vitamin B12	0.7	µg	2.8	µg
Folate	32.4	µg	130.5	µg
Pantothenic Acid	0.5	mg	2	mg
Biotin	3.9	µg	15.7	µg
Vitamin C	13.1	mg	52.8	mg
Ash	0.3	g	1.3	g
Folic Acid	0	µg	0	µg
Food Folate	21	µg	84.7	µg
Dietary Folate Equivalents	21	µg	84.7	µg
Choline	1.8	µg	7.4	µg
Vitamin A IU	1129.6	IU	4554.5	IU
Vitamin A RAE	84.4	µg	340.3	µg
Beta Cryptoxanthin	6	µg	24.3	µg
Lycopene	trace	µg	trace	µg
Lutein Zeaxanthin	933.2	mg	3.7626	mg
Vitamin K	60.3	µg	243.1	µg