OFFICIAL

PROJECT NUMBER: NE172 (Rev.)

TITLE: Nutritional Risk and Antioxidant Status in the Elderly

DURATION: October 1, 1999 to September 30, 2004

JUSTIFICATION:

High intakes of fruits and vegetables are associated with decreased risk of chronic diseases, especially those related to oxidative stress. However, fruit and vegetable consumption is low in many older adults. Identification of the components in food that confer protection is important. Assessment of nutrition status as related to low fruit and vegetable intake, by the use of biochemical markers and dietary assessment tools, coordinated with appropriate intervention and education strategies is imperative for decreasing disease risk in the elderly.

Oxidative stress (OS) is defined as an imbalance between oxidants and antioxidants in favor of the former. The resultant is oxidative damage to molecules such as DNA, lipids and proteins, is implicated in many human diseases. OS is an important etiologic factor in the pathogenesis of the common diseases of aging including cardiovascular diseases, (1,2), ocular diseases such as cataracts (3,4) age-related macular degeneration (AMD) (5,6), neurologic disorders such as Parkinson's Disease (7) and some cancers (8-10).

Many of these diseases associated with oxidative stress can be extremely debilitating, with profound impact on the quality of life of elderly patients as well as caretakers (11), and are associated with disproportionately high health care costs (12). These are critical issues given the expected growth in both number and proportion of older Americans well into the 21st century (13). All of these factors argue strongly for a better understanding of both causative agents in oxidative stress and also of protective agents (i.e. antioxidant defenses).

Certain foods (e.g. fruits and vegetables) and dietary constituents (e.g. antioxidants) found in fruits and vegetables may play a protective role in reducing oxidative damage that occurs throughout the life span (14-16). The consumption of fruits and vegetables has been associated with lower incidence and lower morbidity and mortality rates of cancer (17,18), heart disease mortality (19,20) and hypertension.

The studies to date reveal some fundamental gaps that will be addressed in the proposed research. These include questions regarding the effects of antioxidant compounds and the antioxidant capacity of fruits and vegetables. Prior and his colleagues have conducted a series of studies addressing these issues (21-24) which is believed to represent the first attempt to measure the total antioxidant capacity of fruits and vegetables (23,25). More work is necessary to evaluate the effect of genetics (i.e. species, varieties) and environmental factors (e.g. maturity at harvest) on antioxidant capacity of foods.

Despite the importance of fruits and vegetables to the health of older adults, many are not consuming adequate amounts. Furthermore, there are little data on interventions designed to increase fruit and vegetable consumption in the elderly. To target appropriate interventions, research is needed on elder food practices and food group patterns that are related to antioxidant intake and status, as well as perceptions of barriers and benefits to changing dietary intake.

Among the research issues that still need to be addressed are: 1) evaluation of biomarkers of nutritional risk, with an emphasis on antioxidant status, 2) improvement of dietary assessment methods and screening protocols to identify nutritional risk, 3) elaboration of the relationships between dietary measures and biochemical markers in identifying health risk and susceptibility to disease, and 4) development and adaptation of educational approaches to reduce nutritional risk. Addressing these research questions will require multiple perspectives that integrate food intake behavior and biomarker research. Since nutrition is a national priority for agriculture, extension and research, a cooperative regional approach to continue to address the nutritional needs of older adults is timely and important.

RELATED CURRENT AND PREVIOUS WORK:

The following review identifies research needs in the areas of oxidative stress and disease; diet, antioxidants, and disease; dietary intake methods; and educational interventions/approaches.

Oxidative Stress and Disease

Oxidative stress (0S) is defined as an imbalance between oxidants and antioxidants in favor of the former, resulting in oxidative damage to molecules such as DNA, lipids and proteins. OS can arise from both extrametabolic (e.g. pollution, radiation, toxins) and metabolic sources. The detrimental impact of OS is attributed to toxic intermediate compounds generated from oxygen during normal metabolism. Oxygen is reduced with the addition of electrons. During this process, unstable compounds including free radicals are generated (14-16, 26-29).

The oxidative damage of free radicals appears to be critical in the etiology of various age-related diseases. For example, a large body of evidence is available suggesting that oxidative damage plays an important role in the pathogenesis of atherosclerosis and neurodegenerative disease (26,27). Animal studies have provided further evidence by suggesting that free radicals may promote thrombosis, directly damage vascular cells and other tissues, and interfere with vasomotor regulation (28,29) with the clinical sequelae of myocardial infarction and ischemic stroke. In addition, vascular disorders have been implicated in the most frequent type of dementia, Alzheimer's disease (30). The eye, exposed to various oxidizing metabolic products, is susceptible to oxidative damage (31). Major proteins of eye lens' are vulnerable to oxidative stress with consequent opacities in the lens. The eye's retina contains polyunsaturated fatty acids that are susceptible to photo-oxidative damage as well as other oxidative-stress sensitive compounds located in the macula, the central part of the retina.

Diet, Antioxidants, and Disease

There is a growing body of evidence from human and animal studies that fruit and vegetable consumption reduces risk for various age-related diseases (19,21,32) including heart and vascular disease (20,33), some cancers (17,18,32) and eye diseases (34,35). The studies to date raise some fundamental questions such as the mechanism of the protective role of antioxidant compounds and the antioxidant contribution of specific fruits/vegetables. Prior and colleagues at the Tufts Human Nutrition Research Center on Aging (HNRCA) have developed an effective way to evaluate total antioxidant capacity of foods, the Oxygen Radical Absorbance

Capacity (ORAC) Assay (25,36). The assay provides an effective way to evaluate the total antioxidant capacity in fruits and vegetables. It combines both inhibition time and inhibition degree of the free radical or oxidant action by an antioxidant into a single quantity using an area under the curve technique for quantitation of the data.

Studies from HNRCA represent the first attempt to measure total antioxidant capacity of fruits and vegetables (23,24,37,38). The antioxidant capacity of common fruits and vegetables, and drinks including green and black teas, commercial fruit juices and wines, were measured with the automated ORAC assay using a preaxial radical generator ($ORAC_{ROO}$). Based upon the weight of edible portion, prunes, raisins, blueberries, cranberries, and blackberries had the highest antioxidant capacity. Strawberries, raspberries, garlic, kale, and spinach had an medium antioxidant capacity while brussel sprouts, alfalfa sprouts, plums, broccoli flowers, beets, oranges, red grapes, red bell pepper, cherries, and kiwifruit had a lower antioxidant capacity. More work is necessary to evaluate the effect of genetics (i.e., species, varieties) and environmental factors (e.g. maturity at harvest) on antioxidant capacity of fruits and vegetables.

While increased consumption of fruits and vegetables has been associated with protection against various age-related diseases, (39-43) it is not known which dietary constituents are responsible for this association. It is often assumed that well-characterized antioxidants including vitamins C and E, or β–carotene contribute to the protection (44-51). However, the results from intervention trials have not been conclusive regarding the protection from supplementation with these antioxidants (52-55). Three classes of antioxidant compounds will be considered.

Flavonoids as Antioxidants: The beneficial effects of a high intake of fruits and vegetables on the risk of diseases may not be exclusively a result of the action of antioxidants such as vitamins E and C, or β–carotene. The effect may be a result of other antioxidant phenolic phytonutrients or perhaps from a concerted action by a combination of different antioxidants present in these foods. Results by Cao and coworkers (23,24,37,38) have shown that many fruits and vegetables have strong antioxidant capacities, and that this activity is due mainly to non-vitamin C antioxidant phytonutrients. Among these phytonutrients, flavonoid compounds including flavonones, catechins, and anthocyanins are important components of this group of natural antioxidants (56-61). Anthocyanins are widely distributed in fruits and vegetables. The intake of anthocyanins in humans has been estimated to be 180-215 mg/day in the U.S. (62). The intake of other flavonoids including quercetin, kaempferol, myricetin, apigenin and luteolin has been estimated to be 23 mg/day (63, 64).

Carotenoids as Antioxidants and Age Related Macular Degeneration (AMD): AMD is a serious eye disorder that gradually destroys central vision, which is the ability to read and see straight ahead. In the past decade, it has become increasingly apparent that the clinical entity known as macular degeneration is actually a heterogeneous group of disorders with multiple pathophysical mechanisms. (65). The phrase "age-related" is associated with macular degeneration because data show that the risk of AMD dramatically increases after age 60. It is estimated that AMD caused visual impairment in approximately 1.7 million of the 34 million Americans over the age of 65. (66). Macular degeneration is now considered to be the leading

cause of vision loss in the elderly and that AMD will cause more cases of blindness than glaucoma and diabetic retinopathy combined. (65).

In some studies, it has been shown that high levels of dietary or blood carotenoids are associated with a decreased risk of macular degeneration. In other studies, high intake of dietary carotenoids and/or high levels of circulating carotenoids are associated with high levels of macular pigment. (67, 68). Macular pigment is composed of the carotenoids lutein and zeaxanthin which are of dietary origin. Macular pigment, which can be measured non-invasively, may serve as a biomarker of nutrient intake and may therefore reflect long term nutrient status. Several animal studies have shown a relationship between dietary antioxidants and the integrity of the retina. Although there have been few human investigations, a cross-sectional study using food frequency questionnaires found that subjects with high intakes of carotenoid-rich fruits and vegetables had about a 40% lower risk of AMD (69). In a recent case-control study, Seddon et al. (70) reported that a higher dietary intake of carotenoids, particularly lutein and zeaxanthin, was associated with a lower risk of AMD. Foods that are rich sources of lutein and zeaxanthin include leafy green vegetables such as spinach, kale, collards, chard, and turnip and mustard greens.

In addition to dietary intake of carotenoids, the measurement of macular pigment in the eye may be a biomarker of disease risk. Since ORAC is designed to determine antioxidant levels, if there is a correlation between macular pigment and ORAC, then macular pigment may represent one estimate of antioxidant status. For many, macular pigment is modifiable through dietary intervention. Understanding the relationships between these parameters may help us design appropriate interventions to reduce risk of AMD in the elderly.

Vitamin E as an Antioxidant: Vitamin E (tocopherol) is the major lipid-soluble, radical trapping antioxidant in mammalian membranes and probably the most important antioxidant in lipoproteins. Oxidation of plasma lipoproteins is a suspected causative agent in arterial disease. Free-radical (or non-radical) reactive oxygen species-induced oxidation of membrane constituents or nucleic acids is thought to contribute to the etiology of several other chronic degenerative conditions, including carcinogenesis, inflammatory diseases, and aging (71).

In an effort to quantify vitamin E intake, a vitamin E determination study is proposed. The project would involve quantification of known urinary metabolites of the major tocopherol vitamers, α-tocopherol (α-TOH) and γ-tocopherol (γ-TOH), in urine of the target elderly populations identified for study. These metabolites include (a) α-tocopheronolactone (Simon's metabolite), a quinone-lactone derivative of α-TOH hereafter referred to as α-quinone-lactone (72); (b) 2,5,7,8-tetramethyl-2-(ß-carboxyethy)-6-hydroxychroman, or α-CEHC, hereafter referred to as α-chroman-acid (73,74); and (c) 2,7,8-trimethyl-2-(ß-carboxyethy)-6-hydroxychroman, or γ-CEHC, hereafter referred to as γ-chroman-acid (75). All three metabolites appear to be excreted largely (if not entirely) as glucuronide conjugates, and can be measured in urine in the absence of vitamin E supplementation using sensitive GC-MS analyses (76).

In the US., intake of γ-TOH exceeds that of α-TOH, due in part to common use of corn and soybean oils (salad, shortenings, frying oils) or their products such as margarine and mayonnaise. While these oils contain predominantly γ-TOH, they are also important dietary

sources of α-tocopherol (77). However, plasma and tissue levels of γ-TOH are usually only about 20% that of α-TOH (78) despite their similar absorption efficiencies (79). We have recently found that the majority of dietary γ-tocopherol can be accounted for in the urinary excretion of γ-chroman acid (80). Consequently, urinary γ-chroman acid may be a good marker of dietary intake of the common vegetable oils, which are often consumed as "hidden" fats and therefore difficult to estimate by food intake questionnaires. Also, since these same oils are also major sources of α-TOH, this marker may also be used to estimate α-TOH intake from these oils. In fruits and vegetables the major vitamin E vitamer is α-TOH. While only a small proportion of daily intake of α-TOH is recoverable in 24-hr urine specimens as the α-chroman acid catabolite, this substance may nonetheless be a useful marker of α-TOH intake. With the measurement of these metabolites we expect to obtain a more comprehensive picture of vitamin E intake, status and metabolic utilization in the target populations, and to further evaluate the potential of these urinary metabolites as non-invasive markers of vitamin E exposure and utilization.

Dietary Intake Methods:

Data from NE-172 investigators and others have shown that blood concentrations of antioxidants contained in fruits and vegetables are related to dietary intakes of these foods (21,81,82). Dietary intake studies are thus critical in a comprehensive analysis of antioxidant capacity and age-related diseases.

Most Americans have intakes of fruits and vegetables below the recommended amounts and fail to meet guidelines set by the U.S. Department of Agriculture's Food Guide Pyramid (83) or the 5 A Day for Better Health Program (85-90). This agrees with data available from NE-172 investigations on food group consumption by older adults as well as from the National Nutrition Monitoring System (90-91). There are several questions that remain to be answered. One of these relates to the influence the type of dietary assessment used to estimate fruit and vegetable intake. Differences in estimates of fruit but not vegetable intake by older women when comparing food frequency and 24-hour recall data have been reported (92). Similarly, the choice of instrument affects estimates of fruit and vegetable intake in younger adults (93). Considerable attention has been directed to diet assessment methods, sources of error, and statistical analysis of data. This is reflected in several review articles (94-98), a National Center for Health Statistics Consensus Workshop on Dietary Assessment (99) and international conferences on dietary assessment methodology (100-102). One focus of the papers and workshops on diet assessment is the selection of a method that is appropriate for the intended purpose of the study. Twenty-four hour recalls are preferred when information is needed about actual intake. However, errors in memory and knowledge about what is eaten, as well as socially desirable responses and quantification of how much is eaten will affect the accuracy of dietary data. Food frequency methods are widely used to estimate usual intakes. They, too, are subject to errors of memory. Longer food frequency questionnaires may be unreasonably time consuming and cause fatigue in older adults leading to errors.

Valid and reliable dietary intake measures are needed to assess the need for interventions and to link diet with biochemical indicators of disease risk. To reduce respondent burden and increase quality of data obtained, it is important to use dietary collection instruments that are accurate, reproducible, and rapid. Researchers in NE-172 have studied the use of food frequency

questionnaires (FFQs), the Nutrition Screening Initiative Level I and II screens, and other instruments to assess nutritional risk in older adults. FFQs that omit portion size information, when compared with the semiquantitative format, did not result in significant bias in estimating nutrient intake or nutritional quality in older women (103). However, it is not known if food frequency questionnaires can adequately estimate fruit/vegetable or antioxidant intake if portion sizes are not taken into account, or if other, more rapid, tools can predict risk of low antioxidant status. Furthermore, traditional food frequency questionnaires may not aggregate foods according to antioxidant capacity. Thus, further work is necessary on food group intakes as determined by each type of method and verified with biomarkers.

Education Interventions/Approaches

Despite the importance of fruits and vegetables to health of older adults, there are little data on interventions to increase fruit/vegetable consumption in this population. To target appropriate interventions, research is needed on elder food practices and food group patterns that are related to antioxidant intake and status, nutritional knowledge and attitudes, as well as perceptions of barriers and benefits to changing dietary intake.

While researchers in this regional project and others have identified the barriers to fruit and vegetable intake in younger adults (104), data on older populations are not available. Similarly, interventions to increase fruit and vegetable intake in older adults are not widely available. Although interventions from this region and others targeting populations at worksites, WIC sites, schools and churches have been researched for effectiveness in increasing consumption (105,106), there are limited studies of targeted research-based interventions in older populations.

Numerous research issues need to be addressed: 1) evaluation of biomarkers of nutritional risk, with an emphasis on antioxidant status, 2) improvement of dietary assessment methods and screening protocols to identify nutritional risk, 3) elaboration of the relationships between dietary measures and biochemical markers in identifying health risk and susceptibility to disease, and 4) development and adaptation of educational approaches to reduce nutritional risk. Addressing these research questions will require multiple perspectives that integrate dietary behaviors and biomarker research. Results from this research will lead to a comprehensive understanding of nutritional risk and antioxidant intakes in older adults.

This project is not duplicative of other studies to date. A search (11/4/98) of the CRIS database, using the keywords *elderly, nutrition education, nutrition status, biomarkers, antioxidants, dietary assessment methodology* retrieved over 150 studies. There were no duplications of the research proposed while over 45 studies in the CRIS report were directly supervised and related to the researchers collaborating on this (NE-172) project.

OBJECTIVES:

1. To evaluate biomarkers of nutritional risk, with an emphasis on antioxidant status.
2. To improve dietary intake methods and screening protocols to identify risk in the elderly.
3. To estimate the relationships between dietary assessment and biochemical markers.
4. To develop and adapt educational approaches to reduce nutritional risk.

PROCEDURES:

Objective 1: To evaluate biomarkers of nutritional risk, with an emphasis on antioxidant status.

A common research interest of the participating stations has been the assessment of antioxidant/oxidant status. In the previous project, the work focused on the biochemical markers with little associated dietary information of antioxidant intake. We will include studies of biochemical parameters in relation to dietary information of antioxidant/oxidant status through the joint efforts of the different stations. A flavonoid database of foods is being developed at Tufts and will be incorporated into a food composition database that will allow estimates of intakes of flavonoids. In addition, investigators at USDA-HNRCA have analyzed fruits and vegetables for total antioxidant capacity. These data also will be incorporated into a database that estimates the total of antioxidant capacity of fruit and vegetable intake can be determined in the studies to be described under this objective.

Experiment 1: Determinants of macular pigment density as a biomarker of antioxidant status and disease risk.

The pigment located in the foveal region of the retina, referred to as macular pigment (MP) is composed of the carotenoids lutein and zeaxanthin. Since humans do not synthesize carotenoids, these pigments must originate from the diet. The pigment can be measured in the eye psychophysically using heterochromatic flicker photometry. This technique provides a measure of macular pigment optical density (MPOD) that may serve as a biomarker for carotenoid and antioxidant status, dietary intake, and as a measure of disease risk (67, 107). Factors that influence MP have been studied; however, the relationship between MP, disease risk and diet is still to be established.

In NH, the instrumentation for measuring MP is available. The goal of the following experiment is to determine if MP serves as a biomarker for antioxidant status and to determine how well several assessment tools determine antioxidant status. Additional insight into the determinants of macular pigment will be gained. Seventy-five subjects, aged 55 to 65, will be recruited to participate in the determination of MP. A comprehensive health risk questionnaire will be collected. Each subject will donate a fasting blood sample. The blood will be aliquoted and stored for carotenoid (NH), lipoproteins (CT), LDL subfractionation and ORAC (MA) assessment. Urine samples will be collected for the analysis of vitamin E metabolites (NY).

The Health History and Habits questionnaire (Block), 24-hour recalls and/or other selected instruments will be used to collect dietary information. A validated fruit and vegetable intake questionnaire and dietary methods from CT, MD, ME, RI and MA will be compared to other measurements in this analysis to test for the adequacy of assessing antioxidant intake and status.

Experiment 2: Effects of increasing foods high in antioxidant capacity (primarily food flavonoids) as assessed by the Oxygen Radical Absorbance Capacity (ORAC) assay on biomarkers of antioxidant status.

Elderly subjects will be recruited and assigned to three treatment groups such that two

levels of antioxidant capacity intake are achieved through varying fruit and vegetable consumption. The control group will be subjects consuming no more than 2-3 servings of fruits and vegetables per day (1200-1600 μmol Trolox equivalents/day). In the other 2 treatments, antioxidant capacity intake will be increased up to 7000 μmol Trolox equivalents/day by increasing the intake of selected fruits and vegetables based upon ORAC content of these foods (23,24). The two treatments will be developed so that foods consumed are 1) high in antioxidant capacity, including carotenoids and 2) high in antioxidant capacity but relatively low in carotenoids. Subjects will be studied over a 2 month period of time to assess changes in antioxidant status with the increased intake of antioxidants.

Biochemical parameters which will be assessed in experiments 1 and 2 include plasma total antioxidant capacity measured as ORAC, individual antioxidants (vitamins E and C, carotenoids), uric acid, bilirubin and albumin, and parameters of lipid peroxidation (MDA, 4-HNE, cholesterol hydroperoxides, isoprostane) (USDA-HNRCA; NH; CT). Urine samples (24 hr.) will be collected to measure vitamin E metabolites as a marker of vitamin E intake (NY). ORAC and macular pigment will be measured psychophysically (NH). This measurement may link plasma carotenoids and antioxidant status to AMD risk.

Experiment 3: Retrospective analyses of plasma biomarkers and antioxidant status.

Retrospective analysis will be conducted using data from the Framingham Heart Study, Cohort 20 (67-93 yr). In the current project, plasma vitamin E, retinol and carotenoids were analyzed in this population (108). In the proposed project investigators at CT will work with those at the HNRCA to study the relationship of macular degeneration to plasma antioxidants. Macular degeneration has been determined in this population by investigators at the National Eye Institute. The major research hypothesis is that incidence of macular degeneration will be related to lower concentrations of plasma carotenoids, lutein and zeaxanthin.

In some populations, including the Framingham Heart Study, cohort plasma carotenoids correlate, in general, to dietary carotenoids and consumption of fruits and vegetables (109-111). Retrospectively, we will test the hypothesis that a) dietary carotenoids and b) plasma carotenoids are related to incidence of macular degeneration using the Framingham Heart Study Cohort 20.

In the study of rural elderly in PA, dietary nutrient intake estimates will be collected using five 24 hour recalls collected over one year and food frequency questionnaires. Blood samples will be analyzed by the USDA-HNRCA for antioxidant capacity (ORAC) and other measures of oxidative stress. The Framingham Heart Study, Cohort 20 (67-93 yr) provides a population to assess plasma ORAC and how plasma relates to dietary ORAC. Additionally, plasma antioxidants, vitamin E and carotenoids, assessed in the current study, will be examined for contributions to plasma ORAC activity/interactions. Determinations of how plasma ORAC in this population is associated with risk for coronary heart incidents, i.e., CHD, cerebrovascular events, will be examined.

Objective 2 - To improve dietary intake assessment methods and screening protocols to identify nutritional risk.

Experiment 1: Identification of factors that predict risk of poor nutrition and low fruit and vegetable antioxidant intake in diverse populations.

Existing commonly-used instruments, such as the Nutrition Screening Initiative, Block/Willett food frequency questionnaires, as well as modification of these screens and measures will be evaluated for their ability to categorize elderly subjects into risk of poor nutrition and low fruit/vegetable/antioxidant intakes. Populations in which these evaluations will be applied include subjects from objective 1, elderly African Americans in MA and DC, older adults in public housing in CT and RI, and elderly in PA, RI, and ME. Refined instruments will be utilized in subsequent studies from the region. Data will be further analyzed to assess predictors of low fruit/vegetable/antioxidant intake, such as food security, social support, environmental access, nutrition attitudes, and food behaviors.

Experiment 2: Exploration of food patterns which may be associated with diet related protection against oxidative stresses.

Diets of older adults collected as a part of the CSFII '94-'96 data set will be examined to identify distinct food patterns which may be linked to protection against oxidative stresses (MA). These dietary data are derived from 2 in-person 24-hour recall interviews collected on more than 15,000 people of all ages. Cluster analysis will be used to characterize these food groups or patterns of consumption. These derived factors will be correlated to fruit/vegetable/antioxidant intake and Food Guide Pyramid servings. Participating stations (MA, MD, ME, PA, CT, and RI) will collect 24-hour recalls and food frequency questionnaires on elderly in the region to test the strength of these food groupings.

Objective 3: To estimate the relationships between dietary assessments and biochemical markers.

The goal is to correlate the dietary intake of vitamin E, vitamin C, carotenoids , and dietary ORAC equivalents with proposed biomarkers including plasma antioxidant capacity, individual serum antioxidants, lipid peroxidation and MPOD. Participating stations include DC, MA, MD, ME, PA, CT, NH, NY and RI (not all measures at all sites).

Experiment 1

We propose to quantify the concentration of each of three vitamin E metabolites in urine collected during the experiments addressing Objectives 1 and 3. Metabolite excretion rates would be compared with estimates of dietary vitamin E intake (total intake, and intake from specific food sources) to investigate the relationship between intake and catabolism/excretion of tocopherols. In addition, metabolite excretion will be compared with plasma α- and γ-tocopherol levels, and to markers of oxidative stress such as isoprostanes, MDA, and cholesterol oxidation products already being assessed by other investigators. The overall objectives are to obtain a more comprehensive picture of vitamin E intake, status and metabolic utilization in the target populations, and to further evaluate the potential of these urinary metabolites as non-invasive markers of vitamin E exposure and utilization.

Experiment 2

For each of the experiments outlined in Objectives 1 and 2 where both dietary intake and biochemical parameters will be assessed or have been assessed, we will test the hypotheses that dietary variables are associated with biochemical markers, e.g., macular pigments, ORAC equivalents, carotenoids, etc.

From the current study we know that some plasma carotenoids correlate to dietary carotenoids and consumption of fruits and vegetables (109). Retrospectively we will test the hypothesis that a) dietary carotenoids and b) plasma carotenoids are related to incidence of macular degeneration using the Framingham Heart Study Cohort 20.

Statistical analyses will be performed using SAS. For variable means that are not normally distributed, the variables will be transformed. Pearson correlations will be used to estimate associations between dietary variables and plasma measures. To adjust for confounders such as age, BMI and total energy intake, etc., partial correlations will be employed. Intake of most nutrients and other food constituents correlate with energy intake, thus adjustment for this variable allows an assessment of the correlation independent of sample variation in total energy intake, partially adjusting for differences in intake that may be due to body size or activity levels, and for some of the measurement error inherent in the questionnaire (112). For plasma carotenoid concentration we have previously shown that other adjustment variables are related (108).

To estimate the relationship between total number of fruits and vegetables reported as servings consumed per day and each of the dietary variables, we will use Pearson correlations to compare the distribution of each plasma variable with total number of servings of fruits, vegetables, and of combined fruits and vegetables.

These analyses will be the basis for developing educational approaches to reduce nutritional risk, as described in Objective 4.

Objective 4 - To develop and adapt educational approaches to reduce nutritional risk.
Experiment 1: Awareness of and knowledge and attitudes towards age-related macular degeneration (AMD) and other diseases will be determined and related to fruit and vegetable consumption in the elderly.

Elderly subjects in the region, identified under Objectives 1-3, will be surveyed for awareness and knowledge of antioxidants, AMD and other oxidative-related diseases. Attitudes, barriers, and motivators in achieving behavior change will also be identified. Association between knowledge, awareness, attitudes, and other factors with fruit, vegetable, antioxidant, and supplement intake will be determined. Participating stations include MD, MA, CT, RI, DC, NY and ME.

Experiment 2: Development, adaptation, and testing of educational strategies to increase fruit/vegetable/antioxidant intake in community-based populations.

Data obtained from Objective 2, and Objective 4, experiment 1, will be analyzed for educational interventions that will be suitable for different environmental, social, and cultural settings. Community-based educational approaches will be designed and evaluated to increase fruit/vegetable/antioxidant intake as well as knowledge and awareness of oxidative-related diseases. Precedents and facilitators to dietary improvements will be identified using a pretest/posttest design. MD, MA, CT, RI, DC, NY and ME will participate in experimental design and implementation.

EXPECTED OUTCOMES:

We will be studying a number of dietary antioxidants including vitamins Eand C, the carotenoids as well as antioxidant flavonoids. One of thedifficulties in making nutritional recommendations with regard toantioxidants has been the lack of reliable in vivo markers of antioxidant status. One of the outcomes from this research will be the identification of biomarkers which are indicative of antioxidant status and of dietary assessment methods which identify individuals at risk nutritionally for the antioxidant nutrients. With respect to vitamin E, an expected outcome from these studies will be the development of tools which provide for a comprehensive picture of vitamin E intake, status and metabolic utilization in target populations. We expect to be able to utilize specific urinary metabolites as non-invasive markers of vitamin E exposure and utilization. We expect to determine if the intake of dietary carotenoids, or perhaps other dietary antioxidants such as flavonoids or vitamins C or E, are related to the incidence of macular degeneration and other diseases of aging such as cardiovascular disease. As dietary habits tend to be difficult to change, we expect to develop educational approaches that can be used to reduce nutritional risk. The long term outcome of this research will be to provide nutritional information to the general public such that dietary habits can be altered to provide for increased quantities of those foods which maintain antioxidant status in vivo. and thus delay or prevent some of the diseases of aging such as cardiovascular disease, cancer, macular degeneration, etc. Results from this research will lead to a comprehensive understanding of nutritional risk and antioxidant intakes in older adults.

ORGANIZATION:

The Technical Committee will be organized in accordance with the Regional Research Manual of Procedure, October, 1992, pages 21 and 34.

Offices of Technical Committee: Chairperson, Vice-chairperson, Secretary, Regional Administrative Advisor.

Executive Committee: Chairperson, Vice-chairperson, Secretary, Members at Large (1 or more), Regional Advisor.

All voting members of the Technical Committee are eligible for office, regardless of sponsoring agency affiliation. Officers will be elected at the annual meeting with the expectation that the Vice-Chair moves to Chairperson. The chairperson, in consultation with the administrative adviser, notifies the Technical Committee members of the time and place of meetings by mail, prepares the agenda, presides at meetings of the Technical Committee and Executive Committee, and initiates the formulation of ad hoc committees (i.e. nominating committee, publications committee, rewrite committee, etc.). The chairperson is responsible for preparing or supervising preparation of the annual report of the regional project. The secretary records the minutes and performs other duties assigned by the chairperson, the Technical Committee, or the administrative advisor.

A lead research station will direct each study area included under objectives of the proposal. The lead station, in coordination with cooperating stations, will develop methodology, define the study design, coordinate data collection, and analyze all data. The lead station will be responsible for including a biostatistician to serve as a consultant for purposes of sample selection and sample size determinations as well a data analysis. Study protocols will be

submitted to the executive committee. The executive committee will critique overall study design as well as annual work plans for each project.

Each lead station will distribute a current status report to all Technical Committee members before the annual meeting so that the annual meeting can concentrate on review of studies and methods and interpretation and integration of results.

Signature Page

Regional Project Title: Nutritional Risk and Antioxidant Status in the Elderly.

Administrative Advisor — Date: May 4 1999

Chair, Regional Association of Directors — Date: May 4, 1999

Administrator
Cooperative State Research, Extension,
& Education Service — Date: 5/12/99

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Attachments

Project Leaders

Resources

Critical Review

- Work Accomplished
- Degree to which Objectives have been accomplished
- Areas needing further investigation
- Publications

Project Leaders

	Investigators	Specialization
SAES Cooperators		
Connecticut-Storrs	C. Lammi-Keefe	Lipoproteins/Antioxidants
	A. Ferris	Diet Methodology
		Nutrition Education
Maine	R. Cook	Diet Methodology
		Nutrition Education
Maryland	M. Kantor	Nutrition Education
Massachusetts	N. Cohen	Diet Methodology
		Nutrition Education
	M. Laus	Diet Methodology
New Hampshire	J. Curran-Celentano	Carotenoids/Macular pigment
		Nutrition Education
New York	R. Parker	Antioxidants
Pennsylvania	H. Smiciklas-Wright	Diet Methodology
		Nutrition Education
	J. Milner	Nutritional Biochemistry
Rhode Island	C. English	Diet Methodology
		Nutrition Education
	N. Fey-Yensan	Diet Methodology
		Nutrition Education
Washington, DC	C. Jiles	Diet Methodology
	W. Rice	Statistics
NON-SAES Cooperators		
Massachusetts		
USDA/HNRC/ARS	R. Prior	Antioxidants/Flavonoids
	E. Schaefer	Lipoproteins/Antioxidants
	K. Tucker	Diet Methodology
	G. Cao	Antioxidants/Flavonoids
Framingham Heart Study	P. Wilson	Lipoproteins
Tufts	J. Dwyer	Diet Methodology
New York		
New York University	J. Gilbride	Diet Methodology

Resources

	Resources			Objectives			
	SY	PY	TY	1	2	3	4
SAES Cooperators							
Connecticut-Storrs	0.2	1.0	0.0	X	X	X	X
Maine	0.4	1.0	0.0	X	X	X	X
Maryland				X	X	X	X
Massachusetts	1.1	0.5	0.0	X	X	X	X
New Hampshire	0.3	1.0	0.0	X		X	
New York	0.1	0.5	0.0	X		X	X
Pennsylvania	0.2	0.0	0.0	X	X	X	
Rhode Island	0.2	1.0	0.0	X	X	X	X
Washington, DC	0.5	0.0	0.0		X	X	X
Total	**3.0**	**5.0**	**0.0**				
NON-SAES Cooperators							
Massachusetts							
USDA/HNRC/ARC	1.0	0.5	0.5	X		X	
Framingham Heart Study				X		X	
Tufts	0.1	1.0	0.0	X		X	
New York							
New York University	0.1	0.1	0.0		X	X	X
Total	**1.2**	**1.6**	**0.5**				

ATTACHMENT #3: PROPOSED TIME FRAME:

	Time Frame for Activities of Project Technical Committee				
	1999/2000	2000/2001	2001/2002	2002/2003	2003/2004
Implementation Activity	YEAR 1	YEAR 2	YEAR 3	YEAR 4	YEAR 5
literature review	X				
conceptual framework	X				
instrumentation	X				
pretesting	X				
sampling frame	X	X			
interviews, coding & editing data		X	X		
initial analyses			X		
management analyses			X	X	X
family functioning analyses			X	X	X
business analyses			X	X	X
family/business interactions			X	X	X
community structure and characteristics			X	X	X
economic and social contributions			X	X	X
dissemination and publication of results			X	X	X