

# Scientific Advisory Committee on Nutrition

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**Paper for Information: Summary of Findings from the Antioxidants in Foods Research Programme Review**

## **Agenda Item 11**

Please see attached paper for information and discussion. Please also see attached annexes.

Annex 1 Roame A

Annex 2 Membership of Steering Group

Annex 3 Conclusions of the Review

**Members are invited to comment upon the conclusions of the Review**

# Scientific Advisory Committee on Nutrition

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## Summary of Findings from the Antioxidants in Foods Research Programme Review

1. Shortly after the establishment of the Food Standards Agency, an overarching review of the Agency Research portfolio and research management systems took place.
2. 34 recommendations arose from this review. The first of these was that the Agency should adopt a research strategy which is aimed at ensuring that it obtains the scientific information that it needs to be able to meet its aims and objectives in a cost effective way.
3. To this end, it was necessary to review individual programmes inherited from MAFF, and evaluate them not only in relation to the original criteria but also in conjunction with the aims and objectives of a new organisation.
4. The Antioxidants in Foods Programme was established over 10 years ago. The anti-oxidant hypothesis postulates that human degenerative processes involve damage from 'free radicals' generated in the presence of oxygen, and that the processes might be slowed or halted by dietary anti-oxidants. The original statement detailing the rationale (ROAME A) for this programme is attached at annex 1. At its core were two forms of observation:
  - that epidemiological studies indicated a strong and consistent association between high intakes of fruits and vegetables and reduced risk of chronic diseases such as cardiovascular disease and cancer and
  - that in vitro studies suggested a range of compounds in fruits and vegetables with antioxidant activity.
5. The programme focused mainly on the development of biological markers (biomarkers) of oxidative damage to lipids, DNA or proteins that might be suitable for use in dietary intervention studies in humans. Some projects addressed supplementation in vitro, and others issues of bioavailability and effects on gene expression. Projects in the programme have mainly considered the effects of vitamins C, E, and other so-called non-nutrients such as flavonoids found in plant foods such as tea, grapes, onions and many other foods.
6. A basic assumption underlying the programme was that if antioxidants can be shown to reduce oxidative damage, then there is likely to be a beneficial effect on disease risk. The review process was intended to address the evidence supporting the anti-oxidant hypothesis.
7. The review of the programme was commissioned in April 2001 and carried out by the British Nutrition Foundation. It reported in June 2002. The project design included a Steering Group, membership of which can be found at annex 2.
8. The Report describes a review which sets in an international context the findings of 52 research projects funded by MAFF and then FSA as part of the programme.

9. The review should also be considered in the context of other recently completed or on-going reviews such as the National Academy of Sciences (USA) review of vitamin C, vitamin E, selenium and carotenoids; the EU-funded EUROFEDA project; the EU-funded ESCODD project; the work of the Food Standards Agency's Expert Group on Vitamins and Minerals; and the review conducted by BNF of the FSA's Optimal Nutrition research programme.
10. One of the programme objectives was to prepare for a sufficiently powered multi-centre intervention study using a number of validated biomarkers of oxidative stress, to establish optimal intakes for vitamins C and E. The BNF was asked to assess whether the work conducted to date provided a robust case to proceed with this objective in view of the considerable financial resources which would be required.
11. A copy of the conclusions of the review is attached at annex 3. In summary, the review recommended that it would not be appropriate to begin a multi-centre intervention trial. More emphasis should perhaps be placed on discovering other possible mechanisms of action for plant constituents which may be protective. More information is needed about bioavailability of these bioactive substances. Many of the studies within the MAFF programme were carried out on healthy individuals, whereas those most likely to show changes of interest might be those with low plasma levels of the substances of interest. The experience gained in the ESCODD collaboration has taught the importance of collaboration to compare and validate different methods of measurement to ensure comparability between laboratories. In line with the Agency's goal of successfully influencing behaviour change, more work is needed on what influences food selection in particular in relation to vegetables. More work on food preparation or processing and subsequent bioavailability may be needed.

**SACN secretariat**  
**June 2002**

# Scientific Advisory Committee on Nutrition

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## Annex 1

### **ROAME A STATEMENT ANTIOXIDANTS IN FOOD (AN04)**

#### **Background**

An analysis of recent research on the links between diet and health identifies a particularly large and growing corpus of scientific data derived from two key areas. First, epidemiological studies which indicate a strong and consistent association between high intakes of fruit and vegetables and reduced risk of cancers and several other chronic diseases. Second, *in vitro* (and, to a lesser extent, *in vivo*) studies which suggest that a range of compounds that occur in fruit and vegetables have potential antioxidant activity. This has led to the so-called 'antioxidant hypothesis', which postulates that many major human degenerative processes involve in their aetiology free radical processes which may be ameliorated in some way by dietary antioxidants (including both nutrients such as vitamins C and E and non-nutrients such as flavonoids).

The 'antioxidant hypothesis' describes a scientifically plausible means of defining a set of dietary behaviours, achievable using foods which form a staple part of the current national diet, which would provide a significant reduction in risk of a range of chronic, degenerative diseases for the general population.

#### **Rationale for a MAFF research programme**

Several industrial sectors are funding research on dietary antioxidants, for example research funded by tea packers and producers into the potential antioxidant effects of flavonoids found in leaf tea. This research tends to have as its aim the ability of the producer of a certain food to make claims regarding the activity of antioxidants in that specific food.

Antioxidants are found in a wide and varied range of foods and their effects in the body, either singly or in combination, are unlikely to be related in a simple fashion to their effects *in vitro*. It is clear that any structured attempt to address the 'antioxidant hypothesis' will require a consideration of both the physiological mechanisms which underpin the hypothesis and the role of the whole diet in providing an optimal amount and combination of dietary antioxidants. No individual food manufacturer or processor, indeed no individual food industry sector, is therefore placed to capture the benefits from

research of this nature in terms of a monetary return on the investment required.

There is, however, the potential for research in this area to lead to concrete public health benefits. A number of estimates suggest that at least a third of the 138,000 deaths (1996 figures) from cancer in England and Wales each year are related to diet. An even greater number of deaths (238,000 in England and Wales in 1996) are reportedly due to cardiovascular disease, for which a substantial, diverse and generally consistent body of evidence indicates a number of aspects of diet to be major risk factors. The 'antioxidant hypothesis' proposes a role for dietary antioxidants in the reduction of risk of cardiovascular disease, some cancers, and several other degenerative diseases.

These diet-related public health problems impose costs some of which, such as increased NHS provision, would be borne by society as a whole rather than by the affected individual. Reducing the prevalence of chronic disease by dietary means therefore has benefits not only for the individual in terms of increased quality of life, but also for society. Consideration of these social benefits is key in proposing a publicly-funded programme of research. The role for Government as a funder of public health research has been recognised as legitimate in successive White Papers on public health.

MAFF, as the Department with lead responsibility for food and the national diet, has the remit to investigate the role of particular foods and the diet as a whole, so far as they may affect biological processes which in turn can influence health and risk of disease. This directed research programme is funded with discrete public health benefits in mind. MAFF is not acting as a proxy customer on behalf of the food industry, but will use the results of the research directly in framing nutrition policy.

### **Policy objectives**

Further reductions to the incidence of cancer and cardiovascular disease remain a priority in the Government's public health programme. The recent consultation paper *Our Healthier Nation* proposes targets for the reduction of the death rates from these illnesses amongst people aged under 65.

The objective of this programme is to provide a robust basis for population-level dietary guidelines on achievable and palatable changes to the UK diet which would optimise the consumption of foods rich in those antioxidants which are found to have benefits. These guidelines would form part of the strategy for meeting targets for reducing cancer and cardiovascular disease.

### **Scientific and technical objectives of the research programme**

The overall objective of this research programme is therefore to test the 'antioxidant hypothesis' and, if the hypothesis is supported, provide

information to help define the optimal level of intake of antioxidants in the UK diet.

*1. To develop and validate methods for the determination of the nature and extent of biologically relevant oxidative damage to DNA, lipids and proteins that may be applied to ex vivo samples of human tissues.*

For dietary antioxidants to contribute towards the maintenance of good health in humans and reduce the risk of degenerative disease, their effects in the body will need to be mediated by an effect on one or more of the classes of macromolecules that define the structures and functions of cells, organs and communication between them. These macromolecules are themselves the subject of attack by reactive oxygen species and the critical effect of antioxidants will be to reduce the impact of these detrimental processes.

This scientific objective is therefore crucial to the overall success of the programme. If we are to identify the levels of antioxidants in the diet which may reduce the risk of disease, we require robust analytical methods which provide evidence for and a measure of the action of antioxidants in humans. These are 'biomarkers of exposure'.

*2. To determine the bioavailability and metabolism of antioxidants at over a range of physiologically-relevant intakes, and how and in what form the antioxidants are delivered to their sites of action*

Information on the kinetics of absorption, distribution, metabolism and excretion of dietary nutrient and non-nutrient antioxidants in human subjects is scarce. There is very little evidence to indicate targeting of antioxidants derived from the diet to specific tissue sites in humans. In view of the current trend among the public for the consumption of antioxidants, it is also necessary to assess the possible consequences of this practice and, specifically, whether any harm may be caused.

*3. To identify the effects of dietary antioxidants on gene expression and other biomarkers of effect.*

The development and validation of methods to determine the level of oxidative damage to DNA provides a series of 'biomarkers of exposure' to indicate the balance between reactive oxygen species and dietary (and endogenous) antioxidants. For these to yield information of use to developing public health policy, further research is required to link 'biomarkers of exposure' with effects that are more closely linked to disease end-points. This covers effects at both the sub-cellular and the tissue level. Key issues to be resolved are:

- how can we differentiate between oxidative DNA damage that is rapidly repaired or functionally silent, and other oxidative DNA damage that is persistent and may have effect on gene transcription and, eventually, on the function of the cell or organ?

- what is the relationship between tissue antioxidant pools, dietary intake and disease?

#### 4. *To prepare for a sufficiently powered multi-centre intervention study*

Considerable progress has been made in the development and validation of suitable biomarkers and analytical methods. To take this research forward, proposals will be sought for projects to address specifically the question of the optimal amount of dietary antioxidants needed for health, using the biomarkers developed under this programme. The overall aim of this programme, to test the 'antioxidant hypothesis', would be met by a sufficiently powered human intervention study which would assess the effects of dietary antioxidants, at concentrations and in combinations that are attainable in a mixed diet, on robust biomarkers of exposure and effect.

Further details of the research funded as part of this programme are given in the attached annex, along with information on the time-scales for achieving each of the scientific sub-objectives. The outputs expected from the programme are considered further in the following section.

#### **Criteria to be used in evaluating the programme**

The overall criteria to be used to evaluate this programme are whether the balance of evidence will be sufficient to define the required level of inclusion of antioxidants in the diet to provide optimal protection, and whether this information is used to develop and promulgate healthy eating advice.

For the purpose of evaluating the programme as part of its review in 2001, the following intermediate criteria will be used:

- Have robust and validated methodologies been developed for the determination of oxidative damage to DNA, lipids and proteins in human tissue samples? Have these methods been critically evaluated (for example, by publication in a reputable peer-reviewed journal)? Are these methods being used further in this programme in projects which assess the effects of antioxidant supplementation on biomarkers of exposure?
- Have robust and validated methodologies been developed for the determination of carotenoids and flavonoids? Are these methods able to distinguish and determine the forms in which they exist in foods and in human tissue samples. Have these methods been used to establish the bioavailability and metabolism of these compounds in humans? Have these methods been critically evaluated (for example, by publication in a reputable peer-reviewed journal)? Are these methods being used further in this programme in relevant projects?

- Has the programme provided evidence to suggest an effect of increased dietary antioxidant intakes on biomarkers of effects for cardiovascular disease, lung function, breast cancer, or other degenerative disease? If so, have these findings been assessed by expert advisory committees?
- Were the results of the programme effectively disseminated to the public and the wider research community?
- Were the results used to provide timely, robust and accurate advice to Ministers in respect of official pronouncements, or in responses to Parliamentary Questions or external enquiries?
- Is the MAFF research programme recognised by international experts as a source of reliable information which has contributed to a better understanding of the field?

### **Potential non-research means of achieving the programme objectives**

As stated above, the overall objective of this research programme is to provide a robust basis for population-level dietary guidelines on achievable and palatable changes to the UK diet which would optimise the consumption of foods rich in those antioxidants which are found to have benefits. In-house paper reviews are limited in what they can achieve. Such reviews may identify potentially fruitful further lines of enquiry, but these can only be proven by devising and testing research questions, which requires sophisticated techniques and a skills base that is not available within the Ministry. No progress could be made in this area without contract research which calls on a very high standard of excellence in the contractors concerned.

### **Potential alternative research means of achieving the programme objectives**

External experts are involved in the specification of forward research requirements through the close involvement in the programme of the current Programme Adviser (Professor A T Diplock) and the discussions with active researchers, including those from overseas and others with no current MAFF funding, that form part of annual programme workshops. Specific research contracts to meet the requirements that are formulated annually are then awarded following open competition and the appraisal of competing research proposals by independent external experts.

We are confident that this approach, particularly the incorporation of independent expert advice at key stages, ensures that the research means employed to address the programme objectives represent the best research option. This view has been confirmed by feedback from scientists active in other European countries and the USA.

### **The potential to buy in research information from other countries or to participate in internationally-funded and organised research**

Several of the issues covered by this programme are equally relevant in other countries, for example robust methodology for the determination of free radical-mediated damage to DNA and the ameliorative effects of dietary antioxidants. Participation in internationally funded and organised research is carried out where appropriate. In particular, the Ministry makes clear in its annual food research requirements document that it will consider providing applicants with additional national support for EU shared cost actions where these accord with the Ministry's own research requirements.

Other issues covered by this programme are particularly relevant to the UK, for example where these relate specifically to national dietary patterns. Available evidence indicates that these are fundamentally different from the established dietary patterns in other countries, for example in terms of the quantity and type of fruit and vegetables consumed which in turn is a fundamental determinant of antioxidant status. Therefore in many cases either our requirements are of no broader international interest, or internationally-funded research is not sufficiently directed or detailed to address our policy needs. In these cases appropriate research can only be funded by the UK.

### **The potential for co-ordination and collaboration with other public or private sector research funders**

The Ministry is an active member of the Diet and Health Funders Group convened by BBSRC. This group aims to co-ordinate priorities, strategies and programmes between all the public sector agencies that fund nutrition research. Links are also being established, through this group, with the research priorities and programmes of the food industry.

This group was considered along with the other, more informal mechanisms of co-ordination by the Agriculture, Food and Fisheries Research Funders Group. This latter group undertook a detailed study of the food area and, in its first report published in June 1997, concluded that publicly funded research programmes in the food area were generally well co-ordinated and that further links had already been established as a result of the latter group's study.

It is inevitable that there will be mismatch of this programme's objectives and its detailed research requirements with the priorities and objectives of other research funders. This is particularly true of industry funders, but also applies to other public sector research funders with a less directed research portfolio. Occasionally, research topics emerge which are relevant to both the policy-directed objectives of MAFF and to other research funders. In such cases, alternative mechanisms exist through which research can be funded, most notably the LINK programme on Eating, Food and Health for which MAFF is a co-sponsor. In these cases, close working links in the MAFF Chief Scientist's

Group between the managers of policy-directed research programmes and LINK programmes should ensure that results of the research are effectively communicated to those responsible for food policy formulation.

As a matter of course, the Ministry actively uses published research funded by other interests to inform policy and to focus its own research objectives. We also encourage researchers wherever appropriate to seek part funding of research from other sources, either in cash or kind.

**Nutrition Unit  
MAFF RSN Division  
May 1999**

## ANNEX

**A BRIEF REVIEW OF QUESTIONS BEING ADDRESSED BY MAFF RESEARCH IN KEY AREAS ANTIOXIDANTS IN FOOD (with target completion dates for activities that are still ongoing)**

The 1996 Review of MAFF Nutrition R&D concluded that future research in this programme should concentrate on human intervention studies *in vivo* to examine the effects of different diets, by using validated biomarkers developed as potential intermediate predictors of disease end points. The scientific and technical objectives of the programme have therefore been refined to the following, with the outputs from objectives 1 to 3 providing the means by which objective 4 may be addressed.

Development and validation of methods for the determination of the nature and extent of oxidative damage to DNA, lipids and proteins

In the initial years of the programme, some of the research investigated *in vitro* effects *in vitro* of dietary antioxidants. Following the 1996 review, research in this area has focused on the following objectives:

- to develop and validate a range of methodologies for direct or indirect determination of the specific nature and amount of oxidative DNA damage in human tissue samples (by late 1999);
- to develop methodology for mapping such damage at the sequence level to biologically relevant gene loci (by mid 2001);
- to develop a robust methodology for measuring isoprostanes as selective biomarkers for damage to fatty acids in human tissue samples and to validate other, more accessible and easily undertaken methods, against this 'gold standard' (by end 2001); and
- to develop and validate a robust methodology for determination of the specific nature and total load of oxidative protein damage in human tissue samples (by end 2000).

The bioavailability, pharmacokinetics and metabolism of dietary antioxidants

Research in this programme initially, and successfully, aimed at developing methods for assessment of the total antioxidant activity of foods. Following the 1996 review, the research has focused on the following objectives:

- to determine the differential bioavailability, utilisation and metabolism of tocopherols in humans *in vivo* (by mid 2001);

- to develop and validate methods for the determination of flavonoids, polyphenols and their conjugates and metabolites in foods and in *ex vivo* samples of human tissue (by end 1999); and
- to investigate the bioavailability and metabolism of flavonoids and carotenoids in human subjects at levels of intake corresponding to the range of likely dietary intakes (by late 2001).

The effects of dietary antioxidants on gene expression and other biomarkers of effect

a) *The relationship between antioxidant intakes, oxidative DNA damage, gene expression and disease progression*

The general questions to be addressed in this area are:

- Can a reduction in total levels of oxidative DNA damage be facilitated by increased dietary antioxidant intakes? (by end 2002)?
- Can the prevention of oxidative damage to certain identified genes be correlated with reductions in the risk of disease (by end 2002)? Specific issues being addressed are:
  - the effects of antioxidants on the expression of vascular genes and their relationship to tissue changes and other 'biomarkers of effect' in cardiovascular disease progression (by mid 2000); and
  - the effects of antioxidants on the expression of genes regulating cell adhesion and communication and their relationship to maintenance of the immune response (by end 2001).
- How might dietary antioxidants regulate key transcription factors and other aspects of the expression of genes that contribute to the defensive mechanisms of intracellular systems (by end 1999)?

b) *The relationship between antioxidant intakes, tissue antioxidant pools and disease*

In the initial years of the programme, some of the research investigated the effects of antioxidants on cellular systems using models *in vitro* of atherosclerosis and cataract formation and, on extension to studies *in vivo*, investigated whether dietary levels of vitamin E could ameliorate the effects of increased oxidative stress that resulted from a diet high in polyunsaturated fatty acids. Following the 1996 review, research in this area has focused on the following questions:

- What are the effects of dietary antioxidants on the extracellular antioxidant pools in the normal human lung, and what are the effects of these antioxidants on the consequences of atmospheric pollution (by late 2001)?

- Can dietary levels of vitamins C and E maintain the normal structure and functions of:
  - lipoproteins and the vascular epithelium, thereby protecting against early changes in the aetiology of cardiovascular disease?
  - platelets, thereby protecting against the formation of blood clots and reducing the risk of sudden myocardial infarct, which includes both an arteriosclerotic and a thrombotic component?
- What are the effects of dietary antioxidants and flavonoids on levels of these compounds in pre- and post-menopausal human breast, and what are the effects of any increased uptake on the balance of breast cell proliferation and cell death (by end 1999)?

#### Preparatory work for multi-centre intervention study

It is hoped that the forthcoming EU Fifth Framework Programme will assist in this goal by offering UK scientists access to first-class international collaborations and funding. To this end, MAFF will fund as part of this programme:

- co-ordinated workshops to discuss the scope of future EU funded antioxidant studies and the scope for UK research groups to participate in collaborations (by end 2000).

The overall aim of this programme remains to test the 'antioxidant hypothesis'. To that end, we will contribute funding as part of this programme towards:

- a large scale, multi-centre, international human intervention study to test the effects of dietary antioxidants, at concentrations and in combinations that are attainable in a mixed diet, on robust biomarkers of exposure and effect (by end 2004).

Note 1: Many of the scientific and technical objectives of this programme, including those already completed, were part of the programme as originally designed in 1991. This ROAME A statement includes those objectives and reflects subsequent developments in the programme. Objectives for which the timescale extends beyond early 2001 will be subject to revision in the light of the programme review then due.

Note 2: The scientific and technical objectives of this research programme are complementary to those of other MAFF nutrition research programmes, particularly those on:

- Optimal Nutrition Status (AN05) which investigates broader issues concerning the bioavailability and biomarkers of adequate status for a range of nutrients; and

- Dietary Lipids (AN02) which is investigating early stages in the aetiology of cardiovascular disease, roles in the prevention of which have been proposed for dietary antioxidants.

# Scientific Advisory Committee on Nutrition

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## Annex 2

### List of Steering Group members

Professor John Arthur (Rowett Research Institute)

Dr Margaret Ashwell (Ashwell Associates)

Dr Peter Hollman (State Institute for Quality Control of Agricultural Products, (RIKILT), Wageningen, The Netherlands)

Professor Malcolm Jackson (University Of Liverpool)

Prof. Norman Krinsky (Tufts University School of Medicine, Boston, USA)

Professor John Mathers (University of Newcastle upon Tyne)

Professor Helmut Sies (Institute for Physiological Chemistry I, University of Dusseldorf, Germany)

Dr Harri Vainio (International Agency for Research on Cancer, Lyon, France)

Professor José Viña (Department of Physiology, Faculty of Medicine, Spain)

Professor Christine Williams (The University of Reading)

Dr David Lindsay (Consultant and Programme Adviser for N04)

# **Chapter 8**

## **Conclusions and recommendations**

## 8.1 Conclusions

It can be concluded that although there is associative evidence from observational and intervention studies in human subjects that a diet rich in plant foods (particularly fruit and vegetables) conveys health benefits, as do high plasma levels of several nutrients found in these foods and known to be antioxidants, there is no evidence that any particular nutrient or class of bioactive substances makes a special contribution. The Steering Group confirmed that there is still a lack of understanding of the mechanisms underpinning the apparent protective effect of plant foods and that, as yet, there is no clear picture of which components are effective and hence no way of predicting whether all or just some plant foods are important in this respect.

To date much attention has focused on the potential for prevention by antioxidants of oxidative damage to DNA, in particular, but also to proteins and lipids. Oxidative damage to these molecules is now recognised as a process that occurs routinely in cells, as well as being associated with disease and ageing processes. Little is yet known about what might be regarded as a normal level of endogenous oxidative damage or what level might signify increased risk of disease. Furthermore, it has often been taken for granted that generation of oxygen and nitrogen free radicals is a detrimental event that should be prevented, but it is now recognised that ROS and RNS function as signalling molecules in cells and may also be important in triggering events such as apoptosis. In this context, there is the potential for the requirements of antioxidant molecules within cells to be very closely linked with critical functions such as signal pathways and gene expression, disruption of which could be detrimental to cell functioning and survival.

The establishment by MAFF of the *Antioxidants in Food* Programme (AN04/N04) was ground-breaking and the research that has arisen from the Programme has been very important as it has focussed attention on disease prevention rather than treatment. The Programme has helped to progress the identification of biomarkers, in particular.

In the wider context, the EU-funded ESCODD project, which included contractors from the AN04/N04 Programme, has helped to clarify reasons for discrepancies in the measurement of oxidative DNA damage; it has helped in the optimisation of detection methods and devising of standard protocols; and has provided a platform for reaching a consensus about the basal level of damage in human cells. As a result, it is now apparent that the results of some of the earlier human studies need to be re-evaluated in the light of awareness of the artefacts produced during sample preparation for chromatographic analysis of oxidative DNA damage. In other words, some of the earlier studies probably over estimated levels of DNA damage as a result of the nature of the sample preparation techniques; on the other hand it is also possible that some of the studies that have produced a low estimate of endogenous damage are underestimates owing to the methodologies used.

Whilst there is now a small number of robust biomarkers, the biological relevance of these (*e.g.* a direct association with a disease) remains an issue. Furthermore, methods need to be validated in biological terms, not just against one another. Whilst attempts to do this were included in the AN04/N04 Research Programme, the expectations associated with these small intervention studies were probably over-ambitious. One way of taking this requirement forward would be to employ a nested case control study within an existing

prospective study, perhaps using lymphocytes. Again, the applicability of measurement of, say, DNA oxidation in lymphocytes to tissue specific disease needs to be verified.

Another useful way forward might be collaborative studies with surgical departments able to supply biopsy material. This would enable site specific studies to be undertaken. In this context, the sensitivity of existing assays may be a relevant concern.

It is apparent from the Review that a focus on antioxidant mechanisms in isolation is no longer appropriate and diversification is needed. It is also clear that interest in measurement of oxidative damage and the impact on this of antioxidants has run ahead of the basic and necessary underpinning research on the functional effects of antioxidants, their bioavailability and their tissue distribution.

A major objective of this Review has been to establish whether or not there is sufficient robust scientific evidence to justify funding a multi-centre intervention trial. It is BNF's view (and that of the Steering Group) that there remain too many unanswered questions to progress to this stage at this point in time.

The areas of uncertainty or difficulty include:

- ◆ Relationship of the biomarkers to disease processes
- ◆ Variability of biomarkers within and between people 'at baseline' owing to internal or external factors, which may be unknown or difficult to control in intervention trials
- ◆ Inability to measure the biomarkers in the tissues of interest because of practical (or ethical) problems in sample collection
- ◆ Suitability of a particular biomarker across populations, *e.g.* in health and in disease
- ◆ Sensitivity of the assays over the range likely to be of importance in 'real life'
- ◆ Identification of the most likely study population in which to identify an effect
- ◆ Requirement for sample sizes which are impractical.

Nevertheless, we believe there is ample scope for a refocused programme of work that concentrates on establishing the functional effects and bioavailability of various plant food components, including the so-called antioxidant nutrients, but which takes a broader view on mechanisms of action, *e.g.* the interactions between dietary factors and the immune system, markers of endothelial damage (*e.g.* ICAM-1, VCAM-1, P-selectin), modulation of Phase I and Phase II enzymes, and effects on gene expression and cell signalling.

The extent of knowledge about the bioavailability of many of the substances of interest is still very limited. For example, little is known about the proportion of the intake of a particular dietary component that reaches the tissues; why some substances, *e.g.* the carotenoids lycopene and zeaxanthin, are actively concentrated in the prostate gland and macular region of the eye respectively; whether particular metabolites are important and their fate.

Future studies in humans should focus on groups with low intakes/status of the substances in question and perhaps those people more likely to be susceptible to oxidative damage (*e.g.* smokers, those with certain polymorphisms, or those with established disease) or more likely to benefit from an increase in supply of the plant constituents (*e.g.* because of previous low intake or because of prior priming with a stressor – a high PUFA intake was

suggested at the Workshop). The Programme has been hampered by its focus on healthy non-smokers and younger people.

Future studies should also clarify safety issues *e.g.* whether or not some antioxidant nutrients act as pro-oxidants *in vivo* in some circumstances.

Although it is the FSA's expressed intention to focus on foods rather than supplements, there may be merit in comparing the effects of foods, plant extracts and supplements so as to compare the effects of the whole food versus components, and to establish whether a dose response exists. (Some believe there is a role for specific animal experiments in this context.)

The following points may be worthy of consideration:

- ◆ Choose a sample from a population (of an appropriate age and gender) who are likely to be responsive, *e.g.* as a result of low intakes of fruits and vegetables or specific 'protective' nutrients. Other aspects of lifestyle may be relevant, *e.g.* smokers are believed to be at enhanced risk of oxidative damage.
- ◆ Use doses of nutrients/other substances that are within the 'physiological' range and allow investigation of a dose response, noting that the use of high doses of substances, to provoke an effect, may be an essential strategy in the early stages of a research programme.
- ◆ Pay attention to soundly based power calculations, use of RCT designs where appropriate and choice of the most informative endpoints.
- ◆ Genotyping may not be essential in all studies but should be considered in all studies. In cases where the evidence for a genetic predisposition to disease or evidence of genotype-diet interactions is very strong, it is essential to design studies with this in mind and to undertake prospective genotyping. In less obvious cases, it should become routine to store DNA for later retrospective analyses.

## 8.2 Recommendations

We recommend that:

1. It would not be appropriate to begin a multi-centre intervention trial at this point in time.
2. The FSA Research Programme should be refocused to consider a wider range of potential mechanisms of action, not just prevention of oxidative damage, with the ultimate aim of improving understanding of whether or not the link between plant food consumption and reduced chronic disease risk is causative, by identifying mechanisms of action for substances within the foods.
3. More emphasis should be placed on studying the bioavailability (absorption, metabolism and turnover, tissue and cellular distribution) of a range of plant derived substances, including polyphenols as well as recognised vitamins and minerals, to establish whether *in vitro* effects are applicable to the *in vivo* situation.
4. More emphasis should also be directed to understanding the functions of plant derived nutrients and other bioactive substances at a tissue and cellular level, and the impact on these (and on bioavailability) of factors such as genotype, age and ill health. A starting point for this work could be a review of the published literature to establish currently available information about a range of key components of plant foods, *e.g.* certain nutrients and classes of polyphenols, including interaction between them, dose response relationships, and groups of the population likely to have low intakes of these.
5. Any human studies should involve subject groups in whom a response might be anticipated, *e.g.* those with low plasma levels of the substance of interest.
6. There is a need for further collaborative studies (perhaps involving EU funding), such as the ESCODD study (DNA oxidation), which compare and validate different methods of measurement of biomarkers. In addition, the EU-funded EUROFEDA study may also provide useful information to aid decision making.
7. In parallel with this, work is needed on the factors that influence successful modification of food selection. There is also an important role for product innovation, particularly in relation to vegetables. (It is clear from work in the USA and recent work in Britain that it is possible to increase fruit consumption to a modest degree, but success with vegetable consumption has been much less.) Perhaps relevant to this is the need for an improved understanding of how food preparation and processing influences the availability of food components for absorption in the human gut.

Such an approach should help the Agency to formulate more specific and focussed dietary messages, both in terms of population sub groups and in terms of specific food items.